Syntheses and Studies of Metaphase-Arresting Pyrimidinones

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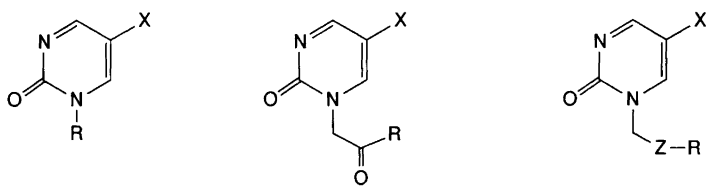
Methods are described for the syntheses of chloromethyl hydroxyalkylphenyl and benzyl ethers, and for the synthesis of bromomethyl phenyl ketone analogs. The hydroxy groups were protected as acetates. The halogenomethyl derivatives have been used for *N*-alkylation of 5-chloro-2(1*H*)-pyrimidinone. The acetyl groups in the products were removed by aminolysis or by enzymatic (pig liver esterase) hydrolysis. The hydroxy derivatives are chemically labile because of polymerization reactions. Adduct formation (1:1) with sodium hydrogensulfite improved the stability. The products are specific inhibitors of the cell cycle in the metaphase. *In vitro* data are given from screening in cultivated Chang liver cells.

Certain *N*-1 substituted 5-halogeno-2(1*H*)-pyrimidinones (**I**) reversibly arrest the mitosis of cells in metaphase, and may be useful in the treatment of diseases caused by uncontrolled, rapidly proliferating cells.[1] Given in a timed, sequential treatment with a phase-specific cytotoxic drug, the aim is to synchronize the cell division cycles so that the normal cells are in an insensitive phase and the abnormal cells are in the sensitive phase, because of kinetic differences, at the time when the phase-specific cytotoxic agent is active. When desired, the deblocking of the cycles must be relatively rapid in order to achieve a high degree of synchronisation. Therefore the blocking agent must be readily removable, and there must be no depot resulting from precipitation of the agent in the body which can serve as a further supply of the active compound. It follows that the active compound must have a fair solubility in water. The best metaphase-arresting properties, however, have been found in derivatives which carry an unsaturated, hydrophobic group on *N*-1. Acidic or basic functions in the *N*-1 group lead to loss of activity. The incompatibility of acidic or basic functions excludes salt formation as a means of solubilization. Instead the compounds have been solubilized by reversible adduct formation with sodium bisulfite or thiols such as 2-mercaptoethanesulfonate in which case the dihydropyrimidine adducts dissolve as sulfonate salts; gradual dissociation of the adducts gives the active metaphase-arresting heteroarene.[2,3] In this report we describe work aimed at the preparation of alcoholic hydroxy analogues in order to improve on the water solubility of active metaphase arrestors.

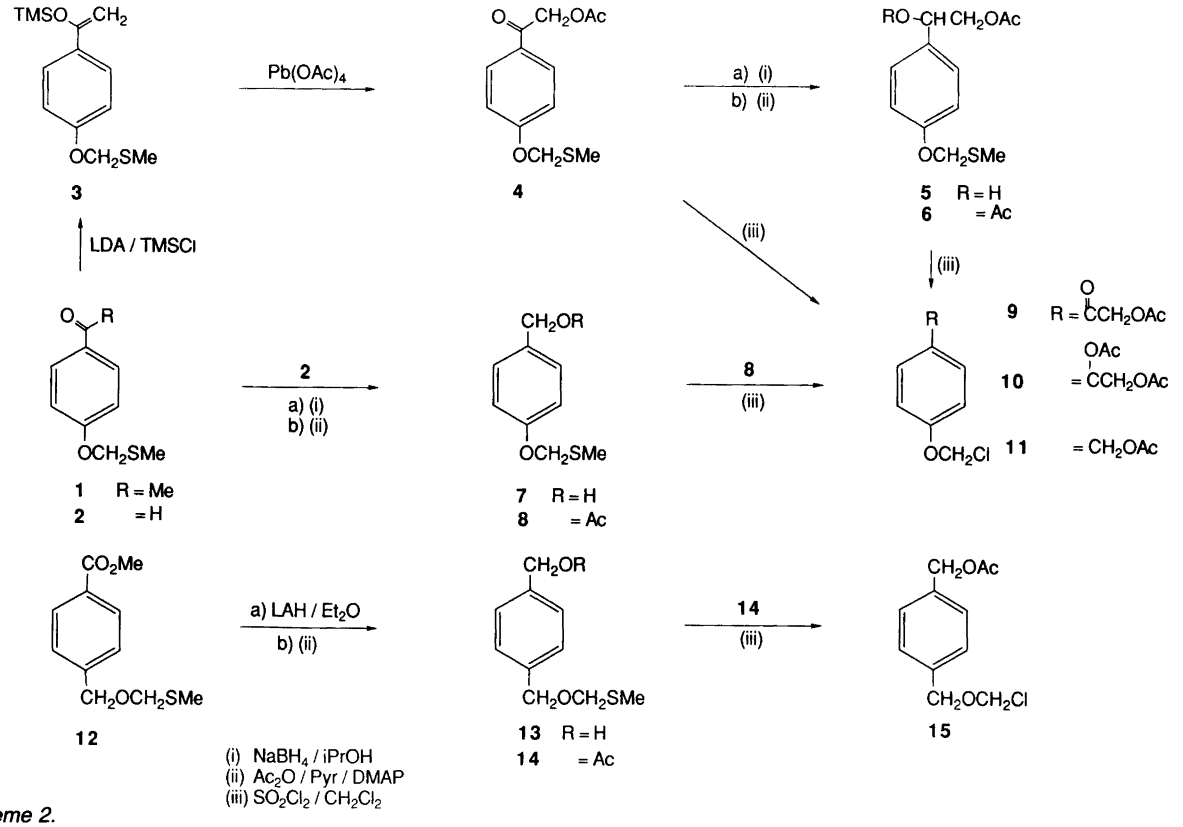
For metaphase inhibitory activity the 2-pyrimidinones should carry a halogen in the 5-position. In most cases a chlorine is preferable, and in this work 5-chloro-2(1*H*)-pyrimidinones substituted on N-1 by either a phenoxymethyl, a benzyloxymethyl group or by a phenacyl group are described. the activity is higher in β-oxo (**II**) or β-oxa (**III**) N-1 substituted derivatives than in corresponding aralkyl derivatives (Scheme 1).[1] This may in part be rationalized as due to negative induction from the β-heteroatom or oxo group onto N-1 with a subsequent effect on the π-electron distribution in the pyrimidine ring. The electronic arrangement in the pyrimidine ring is important since these compounds have been designed to exert their biological function by reversible adduct formation with essential protein thiol groups (*vide infra*).[1]

The compounds prepared carry hydroxy-substituted alkyl groups in the 4-position in the phenyl ring. Corresponding 4-acyl derivatives have previously been reported.[1,2] Their use as intermediates in the synthesis of alkyl hydroxy derivatives has its limitations, however, since the pyrimi-



I II III

Scheme 1.

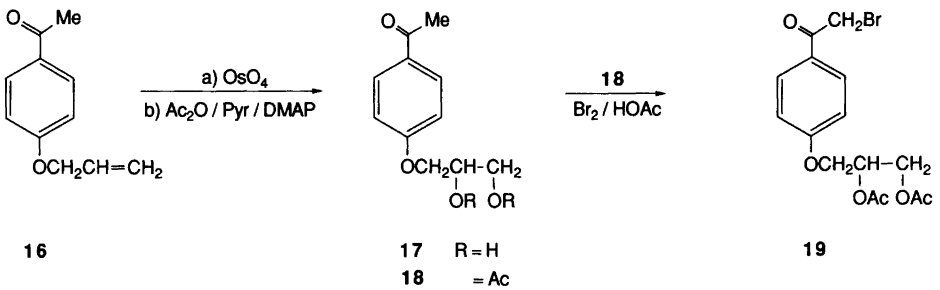


Scheme 2.

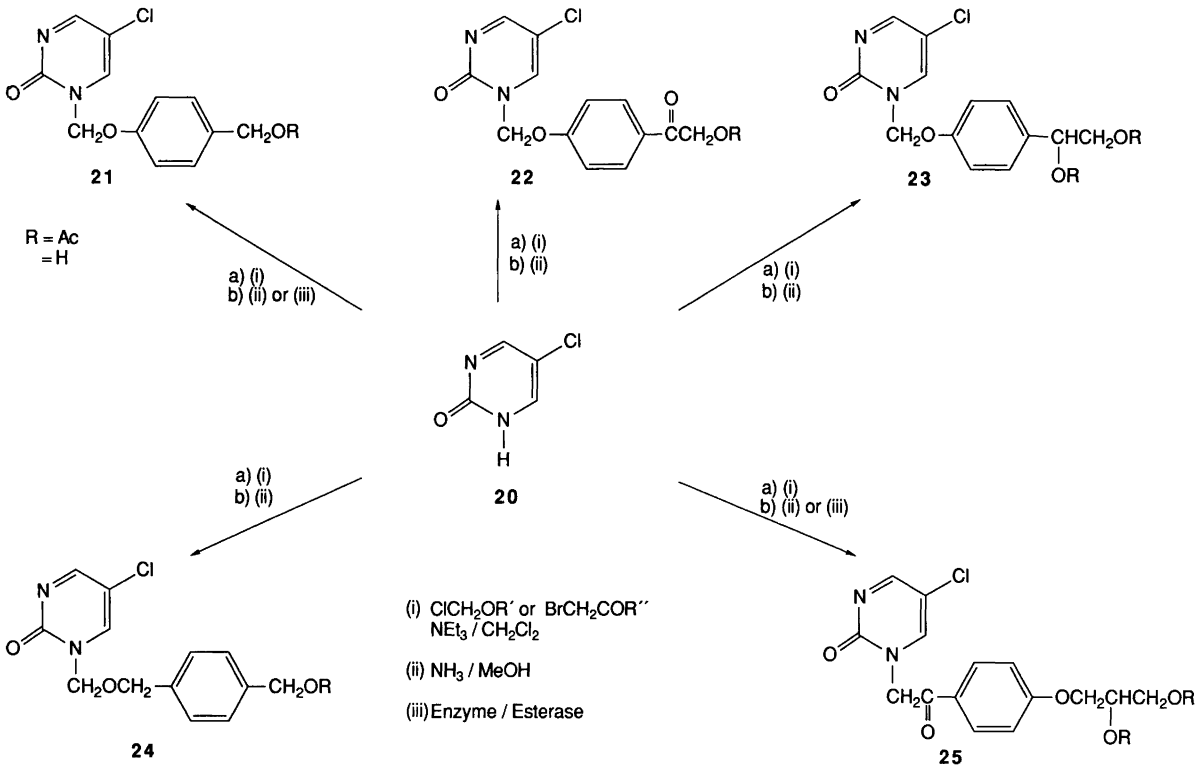
dine ring in the form of a 2-pyrimidinone is sensitive to reductive reactions in the pyrimidine ring, and the ring readily forms adducts with organometallics.[4,5] Therefore the hydroxylated side-chain was prepared separately, and the hydroxy groups protected before attachment of the chain by alkylation to the *N*-1 position in the heterocycle. The alkylating agents in the case of the phenyl- and benzyloxymethyl derivatives were the corresponding chloromethyl ethers. The latter were available from the corresponding thioacetals (**4**, **6**, **8** and **14**) by reactions with sulfuryl chloride, a methodology which we have previously developed and described.[6]

Synthesis of the chloromethyl ether chains to be attached at *N*-1 in the pyrimidinone are shown in Scheme 2. The starting material for the preparation of phenoxymethyl derivatives was 4-hydroxybenzaldehyde or 4-hydroxyacetophenone which was alkylated on the phenolic oxygen with chloromethyl methyl sulfide. Lithiation of the phenacyl derivative **1** and treatment with trimethylsilyl chloride gave the silyl enol ether **3** which was oxidized with lead tetraacetate to the acetoxy ketone **4**. Chemoselective reduction of the ketone oxo group with sodium borohydride furnished the monoacylated vicinal diol **5** which was acetylated to the diacetate **6** with acetic anhydride in pyridine in the presence of 4-*N*,*N*-dimethylaminopyridine (DMAP). For the preparation of the hydroxymethyl derivative **7**, the benzaldehyde **2** was reduced with sodium borohydride and acetylated with the acetic anhydride–pyridine reagent to furnish compound **8**. For the benzyloxymethyl series **15**, methyl 4-hydroxymethylbenzoate was alkylated with chloromethyl methyl sulfide, the product **12** reduced with lithium aluminum hydride (LAH), and the alcohol **13** acetylated to furnish **14**.

In the phenacyl series **19** (Scheme 3) the starting material was 4-allyloxyacetophenone which was dihydroxylated with the osmium tetraoxide–*N*-methylmorpholine *N*-oxide



Scheme 3.



Scheme 4.

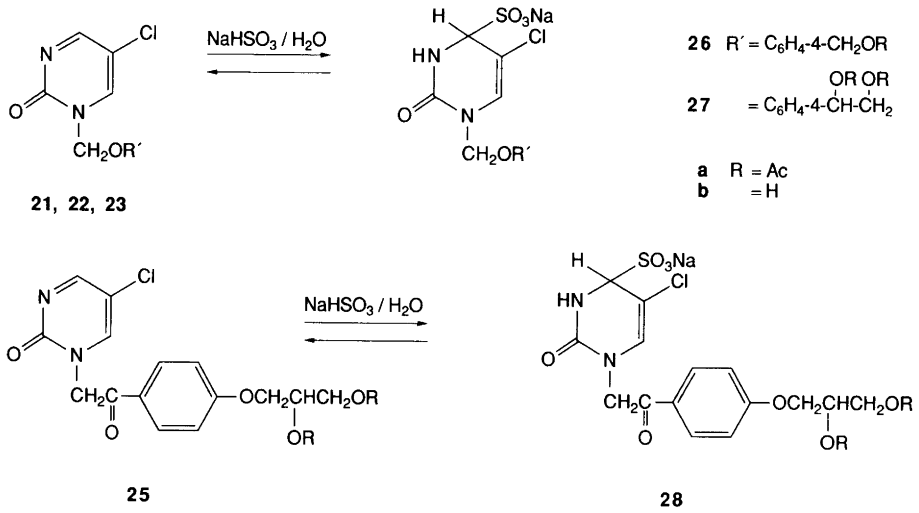
(NMO) reagent,[7] and the diol **17** diacetylated as above. The alkylating agent, the bromomethyl derivative **19**, was prepared from **18** by means of bromine in acetic acid.

Alkylations of the pyrimidinone **20** (Scheme 4) were run on its triethylamine–pyrimidinone complex in dichloromethane. The phenacyl reagent gave exclusive *N*-alkylation. With the harder chloromethyl ether electrophiles minor amounts of the 2-*O*-alkylated isomer were also formed, but this isomer could be removed by chromatography or by crystallization. The *O*-alkylated isomers are inactive as metaphase inhibitors and were therefore not normally isolated.

Difficulties arose in the removal of the protecting acetyl group since the products, the hydroxy compounds, are chemically unstable because of polymerization reactions. The products are sensitive to both acidic and basic conditions. Under acidic conditions ready cleavage of the sidechain occurs at the oxymethyl function, which in its properties is comparable to an acetal. Deacetylation was attempted using either ammonia, sodium methoxide or sodium carbonate in methanol, the ammonia reagent being preferable.

Monohydroxylated derivatives are available by this method, but the dihydroxy derivatives polymerized on attempted isolation (TLC, ^{1}H NMR). All compounds polymerized readily and were only characterized by chromatography and ^{1}H NMR spectroscopy. The phenacyl derivative **25** appears to be chemically more stable than the ether analogue **23** presumably because of the reduced tendency towards nucleophilic addition to the pyrimidine ring. Corresponding derivatives without the hydroxy groups, and the precursor acetates in the present series, do not polymerize. All these compounds form adducts at the electrophilic 4- or 6-position with nucleophilic hydroxy functions, but the adduct formation is reversible. Adduct formation with hydroxylic or amino groups is unavoidable since this class of compound has been designed to exert its biological function by the formation of adducts in a reversible manner (*vide infra*).

The polymerization observed in the hydroxy-containing derivatives can be rationalized as due to intermolecular additions of hydroxy groups to the pyrimidine ring in another molecule. With monohydroxy derivatives a linear oligomeric or polymeric material results; with dihydroxy derivatives a three-dimensional material may form. In the oligomeric or polymeric forms the reversibility of the process to the active monomeric species appears to be reduced, and the dissociation is blocked when the polymeric material is precipitated from the solution. Attempts have been made to avoid polymerizations by deacetylation under neutral conditions using enzyme preparations, esterases and lipases, in buffered media. Pig liver esterase was our best enzyme. The acetate **24** and the propoxy diacetate **25**, but not the ethyl diacetate **23**, were hydrolyzed. The cleavage of the monoacetate **24** was run at 20 °C overnight. Under the same temperature conditions the product from the diacetate **25** contained an additional monoacetate which was not further investigated since the desired deacetylation went to completion when the reaction was run at 30 °C. The products were isolated by lyophilization and subsequent



Scheme 5.

chromatography, and their structures were confirmed by ^{1}H NMR spectroscopy.

Attempts were also made to stabilize the hydroxy derivatives as 1:1 bisulfite adducts (**26–28**). Adduct formation readily took place on treatment of the fully conjugated heterocycle with aqueous sodium bisulfite. The adducts **26a** and **27a** were subjected to aminolysis in methanol to give the corresponding hydroxy derivatives as the 1:1 adducts **26b** and **27b**. It appears preferable, however, to reverse the reaction order. This sequence is described for compounds **21a** and **23a** which were subjected to aminolysis, the solvent removed and the residual mixture of the respective hydroxy derivative and its methanol adduct isomers redissolved in aqueous sodium bisulfite. The bisulfite adduct is formed either by addition to the parent heterocycle or by exchange reactions with the methanol adducts present, and possibly also from polymeric adducts. Mainly the 3,4-adduct was formed, which is the isomer drawn in Scheme 5.

Metaphase arrest. This class of pyrimidinones was designed to exert its biological function by the formation of adducts in a reversible manner with thiol functions in proteins such as tubulin, which is the microtubular protein vital for the formation of the spindle apparatus. When the essential thiol groups in tubulin are blocked, mitosis is temporarily stopped, and continues only when the dissociated pyrimidinone is removed by metabolism. Thiols are bound more strongly in the adduct form than hydroxy derivatives and amines. It is important to control the π-electron deficiency of the pyrimidine ring by suitable substitution, since the π-electron distribution is important for the position of the equilibrium between the free heterocycle and its adducts. When the π-electron deficiency is high, such molecules are isolated as adducts.[8] The Chang line of human liver cells was used to test the activity.[9] Each test substance was dissolved in the culture medium in the highest concentration used, normally 0.5 mM. In each dilution step the concentration of the test compound was halved. Hydrophobic compounds were dissolved in dimethylformamide (DMF) before the medium was added. The concentration of DMF was below the concentration toxic to the cells. The metaphase-arresting activity was accessed 6 h after addition of the pyrimidinone to monolayer cultures of the Chang strain of human liver cells. The number of cells in prophase, metaphase, anaphase, telophase and interphase were compared with controls where cells were present in all phases. When the test substance caused a complete metaphase arrest, and had no other effect on the cell cycle during the 6 h treatment, all cells entering metaphase were arrested and accumulated in this phase. No cells were then present in anaphase or in telophase, and the value recorded in Table 1 is the minimum concentration level where no cells were found in anaphase or telophase and where there was a full accumulation of the cells in metaphase.[10]

The compounds were also tested against leukemia L1210 cells, and it was found that, in general, there is a close agreement between the data from the two test systems.[1] Therefore only the data from the Chang liver cell tests are given in this report. The data for the bisulfite adducts are in most cases the same as for the parent compounds because of ready reversibility of adduct formation.[2]

The acetate **24a** is a highly active compound according to our scale.[1] Its phenoxy analog **21a** is one dilution step less active. The bisulfite **26a**, because of dissociation, showed the same activity as its parent compound **21a**. The activity was reduced by one dilution step in the acetoxymethyl ketone **22a** and further reductions in activity were seen in the two vicinal diacetoxy derivatives **23a** and **25a**. The activities of the monoacetates **21a** and **24a** were retained after hydrolysis to the corresponding hydroxy derivatives **21b** and **24b**, also for the bisulfite adduct **26b**. The hydroxy ketone **22b** and the vicinal dihydroxy derivative **23b**, and its bisulfite adduct **27b** were largely polymerized.

It is concluded that monohydroxy derivatives show similar activities to the parent compounds without the hydroxy group.[1] Introduction of additional hydroxy groups in order to improve the water solubility, however, is not feasible because the products are chemically unstable.

Table 1. Minimum inhibitory concentration (MIC) for complete metaphase arrest of Chang liver cells.

Acetate	MIC/mM[a]	Hydroxy derivatives	MIC/mM[a]
21a (26a)[b]	0.031	**21b (26b)**[b]	0.031
22a	0.063	**22b**[c]	0.5
23a	0.125	**23b**[c] **(27b)**[b]	0.5
24a	0.016	**24b**	0.016
25a (28a)[b]	0.25	**25b**[c]	0.5

[a]The concentration in the medium is halved in each dilution step. [b]Corresponding bisulfite adduct. [c]Fully or partially polymerized.

Experimental

The mass spectra under electron impact conditions were recorded at 70 eV ionizing current. Isobutane was used for chemical ionizing mass spectra (CI); the spectra are presented as *m/z* (% rel. int.). The ^{1}H NMR spectra were recorded at 60, 200 or 300 MHz. The ^{13}C NMR spectra at 75 MHz. The solvent was deuteriochloroform, unless otherwise stated. THF was distilled from sodium–benzophenone.

Screening for metaphase arrest. The Chang line of human cells, established in 1954,[9] was used for the tests. The cells were grown in an E2a culture medium[11] containing human serum (20 %) and horse serum (10 %). Each test substance was dissolved in the medium to be used at the maximum concentration for the testing. When compounds were difficult to dissolve in the medium, they were initially dissolved in DMF and the solution mixed with the medium. The concentration of DMF was below the concentration of DMF toxic to the cells. The medium solutions thus prepared were filtered through a 0.22 μm Millipore bacterial filter, and diluted with the medium to give the desired concentration of the compound for testing. The pH of the medium was adjusted to 7.2. All manipulations of the cells were carried out at 37 °C, and all solutions which were added to the cells had this temperature.

At the start of an experiment, cells from stock cultures after trypsinization were suspended in the medium in a concentration of 100 000 cells per ml. 1 ml of the suspension was added to each of a series of Leighton tubes (16×83 mm; Bellco Glass, Inc., USA which contained a glass strip (35×10.5 mm; Bellco Glass, Inc.) fastened with a chick plasm-embryo extract clot to the flat wall.

The tubes were placed horizontally and kept at 37 °C for 24 h. During this time the cells fastened to the underlayer (the glass strips) and went into exponential growth. The medium was then replaced with new medium. After incubation for another 24 h the strips were covered by an almost confluent sheet of cells in exponential growth.

The medium was then replaced with a medium containing the test substances in the desired concentrations, and the tubes reincubated in the stationary, horizontal position. After incubation for 6 h the strips were removed from the tubes and placed in Carnoy 6 : 3 : 1 fixative and the cells on the strip stained in Boehmer's haematoxylin. The slides were coded before counting. 1 000 cells were counted on each of five slides and the cells assigned to one of the following categories: prophase, metaphase, anaphase, telophase and interphase.

When the test substance caused a complete metaphase arrest and had no other effect on the cell cycle during the 6 h treatment, all cells entering metaphase were arrested and accumulated in this phase, giving a higher count than in the control group. The value recorded in Table 1 is the minimum concentration level where no cells were found in anaphase of telophase and where there was a full accumulation of the cells in the metaphase. The following concentrations were used: 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0.008 and 0.004 mM. In some experiments higher as well as lower concentrations were tested. In all experiments, however, the concentration was halved in each dilution step.

1-Methylthiomethoxy-4-(1-trimethylsilyloxyethenyl)benzene (**3**). Compound **3** was prepared from 4-(methylthiomethoxy)acetophenone[12] using the general procedure described for lithiation with LDA and subsequent treatment with trimethylsilyl chloride;[13] yield 84 %, b.p. 120–125 °C/0.05 mmHg. ^{1}H NMR (300 MHz): δ 0.28 (Me_3Si), 2.26 (SMe), 4.40 (1 H, d, *J* 1.5 Hz), 4.83 (1 H, d, *J* 1.5 Hz), 6.9 and 7.6 (4 H, Ar).

1-Acetoxyacetyl-4-methylthiomethoxybenzene (**4**). Compound **4** was prepared from 1-methylthiomethoxy-4-(1-trimethylsilyloxyethenyl)benzene by oxidation with lead tetraacetate;[14] yield 63 %, m.p. 59 °C (hexane/Et_2O). Anal. $C_{12}H_{14}O_4S$: C, H. ^{1}H NMR (60 MHz): δ 2.15 (3 H, s), 2.21 (3 H, s), 5.10 (2 H, s), 5.18 (2 H, s), 6.9 and 7.9 (4 H, Ar). MS: 254 (M^+, 0.2), 182 (1), 181 (8), 121 (3), 104 (2), 93 (1), 76 (22), 63 (5), 61 (100).

1-(1,2-Diacetoxyethyl)-4-methylthiomethoxybenzene (**6**). Sodium borohydride (0.24 g, mmol) was added at ambient temperature to a solution of 1-acetoxyacetyl-4-methylthiomethoxybenzene (2.05 g, 8.1 mmol) in 2-propanol (25 ml). The mixture was stirred for 2.5 h before water was added and the solution acidified with 1 M HCl. The product was extracted into dichloromethane, washed with saturated sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated. The residue (**5**) was dissolved in pyridine (15 ml), acetic anhydride (8 ml) and DMAP (0.2 g, 1.6 mmol) were added. The mixture was stirred under N_2 at ambient temperature for 24 h before methanol was slowly added and the solution extracted with diethyl ether (×3) The solution was washed with 1 M HCl (×3) and saturated sodium hydrogen carbonate (×2), dried ($MgSO_4$) and evaporated; yield 1.85 g (79 %). ^{1}H NMR (60 MHz): δ 2.08 (3 H, s), 2.10 (3 H, s), 2.25 (SMe), 4.28 (2 H, d, *J* 6 Hz), 5.13 (SCH_2O), 5.97 (1 H, t, *J* 6 Hz), 6.9 and 7.4 (4 H, Ar).

1-Acetoxymethyl-4-methylthiomethoxybenzene (**8**). Compound **8** was prepared from 4-methylthiomethoxybenzaldehyde (**2**)[15] by sodium borohydride reduction as described for the reduction of **4** and the crude product **7** acetylated as described for the preparation of **6**; yield 88%, oily substance. ^{1}H NMR (60 MHz): δ 2.11 (MeCO), 2.28 (SMe), 5.07 (2 H, s), 5.18 (2 H, s), 7.0 and 7.4 (4 H, Ar).

General method for chloromethylation (**9–11**). Sulfuryl chloride (1.35 g, 10 mmol) in dry dichloromethane (20 ml) was added dropwise with stirring over 10 min to a solution of the methylthiomethoxy derivative **4**, **6** or **8** (10 mmol) in dry dichloromethane (30 ml) at ambient temperature. The mixture was stirred for 30 min before the solvent and methanesulfenyl chloride were evaporated at reduced pressure. The crude product thus obtained was used in the subsequent alkylation reactions without further purification.

1-Acetoxyacetyl-4-chloromethoxybenzene (**9**). Compound **9** was isolated in 95% yield (crude). ^{1}H NMR (60 MHz): δ 2.29 (MeCO), 5.35 (CH_2CO), 5.97 ($ClCH_2O$), 7.2 and 8.0 (4 H, Ar).

1-Chloromethoxy-4-(1,2-diacetoxyethyl)benzene (**10**). Compound **10** was isolated in 90% yield (crude). ^{1}H NMR (60 MHz): δ 2.05 (3 H, s), 2.12 (3 H, s), 4.28 (2 H, d, *J* 5 Hz), 5.83 ($ClCH_2O$), 5.95 (1 H, t, *J* 5 Hz), 7.0 and 7.4 (4 H, Ar).

4-Acetoxymethyl-1-chloromethoxybenzene (**11**). Compound **11** was isolated in 93% yield (crude). ^{1}H NMR (60 MHz): δ 2.13 (MeCO), 5.12 (CH_2OAc), 5.96 ($ClCH_2O$), 7.0 and 7.4 (4 H, Ar).

Methyl 4-methylthiomethoxybenzoate (**12**). Compound **12** was prepared from methyl 4-hydroxymethylbenzoate;[16] yield: 77%, b.p. 110–120 °C/0.01 mmHg. ^{1}H NMR (60 MHz): δ 2.20 (SMe), 3.92 (CO_2Me), 4.67 ($-CH_2-$), 4.70 ($-CH_2-$), 7.4 and 8.0 (4 H, Ar).

1-Hydroxymethyl-4-(methylthiomethoxymethyl)benzene (**13**). Methyl 4-methylthiomethoxybenzoate (11.2 g, 49 mmol) was added to a suspension of $LiAlH_4$ (1.07 g, 29 mmol) in diethyl ether (80 ml). The mixture was refluxed for 20 min, cooled and water carefully added, followed by sulfuric acid (10%, 25 ml). The ether layer was separated and the aqueous phase extracted with two additional portions of diethyl ether. The combined ether extracts were dried ($MgSO_4$) and evaporated; yield 9.1 g (94%), m.p. 54 °C. ^{1}H NMR: δ 1.88 (OH), 2.18 (SMe), 4.57 ($-CH_2-$), 4.62 ($-CH_2-$), 4.67 ($-CH_2-$), 7.27 (4 H, Ar).

1-Acetoxymethyl-4-(methylthiomethoxymethyl)benzene (**14**). Acetic anhydride (12 ml) was added to a solution of 4-hydroxymethyl-1-(methylthiomethoxymethyl)benzene (3.4 g, 17.2 mmol) and a catalytic amount of DMAP in pyridine (25 ml). The mixture was stirred under N_2 at ambient temperature for 24 h before methanol was slowly added and the solution extracted with diethyl ether (×3). The solution was washed with 1 M HCl (×3) and saturated sodium hydrogen carbonate (×2), dried ($MgSO_4$) and evaporated; yield 3.25 g (79%), oily substance. Anal. $C_{12}H_{16}O_3S$: C, H. ^{1}H NMR (60 MHz): δ 2.08 (3 H, s), 2.18 (3 H, s), 4.59 (2 H, s), 4.68 (2 H, s), 5.08 (2 H, s), 7.37 (4 H, Ar). MS: 240 (3, *M*), 210 (3), 193 (5), 192 (39), 164 (7), 163 (64), 135 (15), 132 (16), 43 (100).

1-Acetoxymethyl-4-chloromethoxymethylbenzene (**15**). Compound **15** was prepared from 1-acetoxymethyl-4-(methylthiomethoxymethyl)benzene by treatment with sulfuryl chloride as described above (**9–11**); yield 95% (crude). ^{1}H NMR (60 MHz): δ 2.13 (MeCO), 4.80 ($-CH_2-$), 5.16 ($-CH_2-$), 5.67 ($ClCH_2O$), 7.43 (4 H, Ar).

4-Allyloxyacetophenone (**16**). 4-Hydroxyacetophenone (30 g, 0.22 mol) and *t*-BuOK (24.7 g, 0.22 mol) were added to dry DMF (250 ml), the mixture stirred for 10 min, allyl bromide (26.7 g, 0.22 mol) added and the mixture stirred for 16 h at ambient temperature. The DMF was then evaporated off, water was added, the mixture extracted with diethyl ether, the ether solution dried ($MgSO_4$), evaporated and the residual liquid distilled; yield 27.3 g (70%), b.p. 116 °C/0.3 mmHg (lit.[17] 116–117 °C/2 mmHg).

4-(2,3-Dihydroxypropoxy)acetophenone (**17**). *Method A. Hydroxylation of 4-allyloxyacetophenone.* Osmium tetraoxide (catalytic amount) was added to a solution of 4-allyloxyacetophenone (1.0 g, 5.7 mmol) and *N*-methylmorpholine *N*-oxide (0.9 g, 6.6 mmol) in water (40 ml) and THF (20 ml) and the mixture was stirred overnight under N_2. Sodium hydrogen sulfite (0.2 g) and magnesium silicate (2 g) were then added, and the mixture stirred for 10 min and filtered. The filtrate was evaporated and the residue extracted into water. The water solution was extracted with ethyl acetate and the organic phase was dried ($MgSO_4$) and evaporated. The residue was the crude dihydroxy compound; yield 1.05 g (88%), m.p. 95 °C (EtOAc). Anal. $C_{11}H_{14}O_4$: C, H. ^{1}H NMR (300 MHz): δ 2.25 (Me), 3.7 and 4.2 (4 H, $CH_2CHOHCH_2$), 3.85 (1 H, t, *J* 6 Hz, CH_2OH), 4.10 (1 H, d, *J* 6 Hz, $CHOH$), 4.0–4.1 (1 H, m, CH), 7.1 and 8.0 (4 H, Ar). MS: 210 (18, *M*), 195 (11), 178 (15), 163 (38), 136 (17), 121 (100), 93 (16), 77 (13).

Method B. Alkylation of 4-hydroxyacetophenone. 4-Hydroxyacetophenone (10.0 g, 73 mmol) and 1-chloro-2,3-propanediol (8.1 g, 73 mmol) were dissolved in water (100 ml). The pH was adjusted to 10 by addition of 2 M NaOH and was maintained at ca. 10 while the mixture was stirred at 60 °C for 12 h. The mixture was then extracted with ethyl acetate (5×150 ml) and the organic phase was dried ($MgSO_4$) and evaporated. Recrystallization of the crude product from ethyl acetate gave the product in 75% yield (11.5 g).

4-(2,3-Diacetoxypropoxy)acetophenone (**18**). A solution of 4-(2,3-dihydroxypropoxy)acetophenone (1.2 g, 5.7 mmol), acetic acid anhydride (2.16 g, 21 mmol) and DMAP (0.15 g, 1.2 mmol) in pyridine (4 ml) was stirred at ambient temperature overnight. Methanol was then added and the mixture was stirred for 1 h, whereupon water (50 ml) was added, and the mixture extracted with diethyl ether (3×30 ml). The ether phase was shaken with aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated; yield 1.5 g (88%), m.p. 90°C. Anal. $C_{15}H_{18}O_6$: C, H. ^{1}H NMR (300 MHz): δ 2.15 (OAc), 2.18 (OAc), 2.55 (Me), 4.1–4.5 (4 H, m, CH_2CH(OAc)CH_2OAc), 5.4 (CH), 7.0 and 8.0 (4 H, Ar). MS (CI): 295 (100, *M*+1), 253 (2), 235 (4), 160 (5), 159 (64), 121 (4), 43 (2).

4-(2,3-Diacetoxypropoxy)phenacyl bromide (**19**). Bromine (5.43 g, 34 mmol) was added dropwise over 1 h to a solution of 4-(2,3-diacetoxypropoxy)acetophenone (10.0 g, 34 mmol) in acetic acid (50 ml) and the mixture stirred at ambient temperature for 3 h before being poured into ice–water and extracted with chloroform. The combined organic phases washed with aqueous $NaHCO_3$, dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography (toluene–EtOAc 4:1); yield 17.8 g (70%) of an oily material. ^{1}H NMR (300 MHz): δ 2.05 (OAc), 2.10 (OAc), 4.1–4.4 and 5.3 [4 H, CH_2CH(OAc)CH_2OAc], 4.30 (2 H, s, CH_2Br), 5.3 (CH), 6.9 and 7.9 (4 H, Ar). MS(CI): 375/373 (22/25, *M*+1), 315 (12), 313 (11), 296 (6), 295 (30), 235 (6), 160 (7), 159 (100).

*General procedure for alkylation of 5-chloro-2(1*H*)-pyrimidinone* (**20**) *with the α-chloro ethers.* The chloromethyl ether (2.0 mmol) in dichloromethane (4 ml) was added dropwise with stirring under N_2 to a solution which had been prepared from 5-chloro-2(1*H*)-pyrimidinone (261 mg, 2.0 mmol) and triethylamine (0.24 ml, 2.0 mmol) in dichloromethane (20 ml). The reaction mixture was stirred at ambient temperature for 24 h, aqueous NaCl was then added, and the organic layer was separated, dried ($MgSO_4$) and evaporated to give the crude product which was a mixture of the *N*-1 and *O*-alkylated isomers in 80–95% yield. The isomers could be separated by the lower solubility of the *N*-alkylated isomer in diethyl ether or by chromatography. We were only interested in the isolation and purification of the *N*-alkylated isomer.

*1-(4-Acetoxymethylphenoxy)methyl-5-chloro-2(1*H*)-pyrimidine* (**21a**). Yield 44%, m.p. 108°C (iPr_2O). Anal $C_{14}H_{13}ClN_2O_4$: H. Calc. C, 54.46. Found C, 53.30. ^{1}H NMR (300 MHz): 2.08 (MeCO), 5.07 (2 H, s), 7.0 and 7.4 (4 H, Ar), 8.03 (1 H, d, *J* 3 Hz), 8.57 (1 H, d, *J* 3 Hz). ^{13}C NMR: δ 20.8, 65.4, 75.4, 115.4, 130.1, 130.2, 131.0, 141.9, 153.6, 155.1, 166.0, 170.7. MS: 308 (1, *M*), 188 (1), 187 (2), 178 (6), 149 (2), 146 (3), 145 (32), 143 (100).

*4-(Acetoxyacetyl)phenoxymethyl-5-chloro-2(1*H*)-pyrimidinone* (**22a**). Yield 35%, m.p. 81–83°C. Anal. $C_{15}H_{13}ClN_2O_5$: H. Calc. C, 53.50. Found C, 52.48. ^{1}H NMR (200 MHz): δ 2.27 (MeCO). 5.32 (–CH_2–), 6.00 (–CH_2–), 7.2 and 8.0 (4 H, Ar), 7.8–8.2 (3 H, Ar, H-6), 8.70 (H-4, d, *J* 3 Hz). MS: 336 (1, *M*), 266 (1), 265 (10), 264 (4), 263 (31), 145 (35), 143 (100), 121 (35), 116 (21).

*5-Chloro-1-[4-(1,2-diacetoxyethyl)phenoxy]methyl-2-(1*H*)-pyrimidinone* (**23a**). Yield 42%, m.p. 62–65°C. Anal. $C_{17}H_{17}ClN_2O_6$: H. Calc. C, 53.61. Found C, 54.11. ^{1}H NMR (200 MHz): δ 2.02 (3 H, s), 2.10 (3 H, s), 4.28 (2 H, d, *J* 6 Hz), 5.87 (NCH_2O), 5.98 (1 H, t, *J* 6 Hz), 7.0 and 7.4 (4 H, Ar), 7.79 (H-6, d, *J* 3 Hz), 8.58 (H-4, d, *J* 3 Hz). MS(CI): 381 (6, *M*+1), 323 (38), 322 (16), 321 (90), 279 (4), 278 (5), 265 (4), 263 (5), 205 (4), 203 (11), 193 (70), 179 (100).

*1-(4-Acetoxymethylbenzyloxy)methyl-5-chloro-2(1*H*)-pyrimidinone* (**24a**). Yield 63%, m.p. 109°C. Anal. $C_{15}H_{15}ClN_2O_4$: C, H. ^{1}H NMR (300 MHz): δ 2.10 (MeCO), 4.72 (–CH_2–), 5.10 (–CH_2–), 5.43 (NCH_2O), 7.48 (4 H, s), 7.71 (H-6, d *J* 3 Hz), 8.4–8.6 (H-4, br s). MS: 292 (1), 234 (4), 232 (12), 163 (31), 146 (32), 144 (100), 135 (11), 117 (11), 104 (14).

*5-Chloro-1-[4-(2,3-diacetoxypropoxy)phenacyl]-2(1*H*)-pyrimidinone* (**25a**). 5-Chloro-2(1*H*)-pyrimidinone (3.5 g, 27 mmol) and triethylamine (2.7 g, 27 mmol) were stirred together in dry dichloromethane (50 ml) until all the solid had dissolved. A solution of 4-(2,3-acetoxypropoxy)phenacyl bromide (10.1 g, 27 mmol) was added, the mixture stirred overnight, diluted with chloroform (50 ml), washed with water (3×30 ml), dried ($MgSO_4$) and evaporated. The crude product was triturated with diethyl ether and recrystallized from ethyl acetate; yield 9.5 g (83%), m.p. 120°C. Anal. $C_{19}H_{19}ClN_2O_7$: C, H. ^{1}H NMR (300 MHz): δ 2.10 (OAc), 2.15 (OAc), 4.2 and 4.4 [CH_2CH(OAc)CH_2OAc], 5.30 (NCH_2, s), 5.3 (CH, m), 7.0 and 8.0 (4 H, Ar), 7.70 (H-6, d, *J* 4 Hz), 8.58 (H-4, d, *J* 4 Hz). ^{13}C NMR: δ 20.6 (*Me*CO), 20.8 (*Me*CO), 55.0 (CH_2N), 62.1 (CH_2OAc), 66.2 (CH), 69.2 (CH_2), 110.9 (C-5), 110.9, 114.7, 127.6 and 130.5 (Ar), 146.0 (C-6), 154.4 (C-2), 165.6 (C-4), 170.1 (Me*C*O), 170.4 (Me*C*O), 189.1 (CO). MS(CI): 425/423 (48/100, *M*+1), 295 (27), 235 (14), 159 (75), 137 (7), 133 (11), 131 (27).

Aminolysis in the preparation of **21b–25b**. The acetate **21a–25a** (1.0 mmol) was dissolved in methanol (50 ml) saturated with ammonia and the mixture stirred at ambient temperature overnight before it was evaporated at reduced pressure. The residue was a mixture of the free hydroxy compound (**21b–25b**), the methanol adduct and polymeric material. In the case of **21a** the main product was the free hydroxy compound **21b**. [^{1}H NMR (60 MHz): δ 4.62 (CH_2OH), 5.71 (NCH_2), 7.0 and 7.3 (4 H, Ar), 7.90 (H-6, d, *J* 3 Hz), 8.58 (H-4, d, *J* 3 Hz)].

5-Chloro-1-(4-hydroxymethylbenzyloxy)methyl-2(1H)-pyrimidinone (**24b**) *by enzymatic ester hydrolysis.* 1-(4-Acetoxymethylbenzyloxy)methyl-5-chloro-2(1*H*)-pyrimidinone (85 mg, 0.26 mmol) was added to a mixture of 20 mM ammonium carbonate buffer pH 8.4 (100 ml) and pig liver esterase (300 µl, 48 U, Sigma No. E-3128, EC 3.1.1.1) at 20 °C. The mixture was gently stirred for 20 h, filtered, the filtrate lyophilized, the residue extracted with methanol (×3) and the product purified on preparative TLC plates (silica gel) using acetonitrile; yield 57 mg (77 %). ^{1}H NMR (60 MHz, DMSO-d_6): δ 4.50 (s, 2 H), 4.63 (s, 2 H), 5.25 (NCH_2), 7.26 (4 H, Ar), 8.46 (H-6, d, *J* 3 Hz), 8.63 (H-4, d, *J* 3 Hz). MS(CI). 281 (21, *M*+1), 253 (16), 251 (46), 236 (9), 235 (10), 233 (11), 147 (15), 146 (15), 145 (29), 144 (26), 121 (100).

5-Chloro-1-[4-(2,3-dihydroxypropoxy)phenacyl]-2(1H)-pyrimidinone (**25b**) *by enzymatic ester hydrolysis.* 1-[4-(2,3-Acetoxypropoxy)phenacyl]-5-chloro-2(1*H*)pyrimidinone (150 mg, 0.35 mmol) was added to a mixture of 0.1 M sodium phosphate buffer pH 7.5 (300 ml), DMF (33 ml) and pig liver esterase (3 ml, 4800 U, Sigma No. E-3128, EC 3.1.1.1) at 30 °C. The mixture was gently stirred at 30 °C for 20 h, after which the clear solution was lyophilized, the residue extracted with methanol (×3) and the methanol solution evaporated at reduced pressure. The residue was dissolved in 20 % aqueous acetonitrile (20 ml) containing 0.1 % trifluoroacetic acid, and the solution was chromatographed on a C-8 reversed-phase column (Merck, Lobar Fertig Saulen) using the same solvent mixture for the mobile phase. The product eluted after ca. 15 min and was isolated by evaporation of the eluates at reduced pressure; yield 91 mg (67 %). ^{1}H NMR (300 MHz): δ 3.6–4.32 (4 H, m), 7.10 and 7.98 (4 H, Ar), 8.12 (H-6, d, *J* 3 Hz), 8.59 (H-4, d, *J* 3 Hz). When the enzymatic hydrolysis was run at 20 °C for the same time the product was a mixture of **25b** and its monoacetyl derivative (5:1).

Sodium 1-(4-acetoxymethylphenoxy)methyl-5-chloro-2-oxo-1,2,3,4-tetrahydropyrimidine-4-sulfonate (**26a**). 1-(4-Acetoxymethylphenoxy)methyl-5-chloro-2(1*H*)-pyrimidinone (0.92 g, 3.0 mmol) was added to a solution of sodium metabisulfite (0.38 g, 2.0 mmol) in water (30 ml). The pH was adjusted to ca. 7 and the mixture stirred at ambient temperature for 24 h. The reaction mixture was concentrated and cooled and the precipitate was filtered off and washed with diethyl ether; yield 0.90 g (74 %). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.15 (Me), 4.43 (1 H, d, *J* 2.8 Hz), 5.11 (2 H, s), 5.28 (1 H, d, *J* 8.4 Hz), 5.48 (1 H, d, *J* 8.4 Hz), 6.94 (1 H, s), 7.1 and 7.4 (4 H, Ar), 8.79 (1 H, d *J* −2.8 Hz).

Sodium 5-chloro-1-(4-hydroxymethylphenoxy)methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-4-sulfonate (**26b**) *and 5-chloro-1-(4-hydroxymethylphenoxy)methyl-2-oxo-1,2,3,6-tetrahydropyrimidine-6-sulfonate.* 1-(4-Acetoxymethylphenoxy)methyl-5-chloro-2(1*H*)-pyrimidinone (1.0 g, 3.3 mmol) was added to MeOH (100 ml) saturated with ammonia. The mixture was stirred at ambient temperature for 24 h, before the solvent was evaporated off and the residue washed with water (3×10 ml), extracted into a mixture of chloroform containing a little methanol and the solution washed with brine (2×). The dried ($MgSO_4$) solution was evaporated and metabisulfite (0.42 g, 2.1 mmol) in water (25 ml) was added to the residue. The pH was adjusted to ca. 7, the mixture stirred overnight and the clear solution freeze-dried. The product was a mixture of the 4-and 6-sulfonate in the ratio 5:3. ^{1}H NMR (300 MHz, DMSO-d_6) for the 4-isomer **26b**: δ 4.29 (H-4, d, *J* 3.6 Hz), 4.41 (CH_2OH), 5.13 and 5.43 (2 H, AB, *J* 10.0 Hz), 6.79 (H-6, s), 6.9 and 7.2 (4 H, Ar), 7.60 (NH, d, *J* 3.6 Hz). The 6-sulfonate isomer: δ 4.42 (CH_2OH), 4.53 (H-6, s), 5.38 and 5.85 (2 H, AB, *J* 10.0 Hz), 6.34 (H-4, d, *J* 5.4 Hz), 6.9 and 7.2 (4 H, Ar), 8.79 (NH, d, *J* 5.4 Hz).

Sodium 5-chloro-1-[4-(1,2-dihydroxyethyl)phenoxy]methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-4-sulfonate (**27b**) *and 5-chloro-1-[4-(1,2-dihydroxyethyl)phenoxy]methyl-2-oxo-1,2,3,6-tetrahydropyrimidine-6-sulfonate.* 5-Chloro-1-[4-(1,2-dihydroxyethyl)phenoxy]methyl-2(1*H*)-pyrimidinone (0.29 g, 0.74 mmol) was added to MeOH (50 ml) saturated with ammonia. The mixture was stirred at ambient temperature for 24 h, before the solvent was evaporated off and the residue washed with water (3×10 ml) and extracted into a mixture of chloroform containing a little methanol. The solution was then washed with brine (2×). The dried ($MgSO_4$) solution was evaporated and metabisulfite (0.21 g, 1.1 mmol) in water (15 ml) was added to the residue. The pH was adjusted to ca. 7, the mixture stirred overnight and the clear solution freeze-dried. The product was a mixture of the 4- and 6-sulfonates in the ratio 5:2. ^{1}H NMR (300 MHz, D_2O) for the 4-isomer **27b**: δ 3.68 (2 H, d, *J* 6 Hz), 4.73 (1 H, t, *J* 6 Hz), 4.86 (H-4, s), 5.24 (1 H, d, *J* 9 Hz), 5.42 (1 H, d, *J* 9 Hz), 6.71 (H-6, s), 7.0 and 7.3 (4H, Ar). ^{1}H NMR (D_2O) for the 6-isomer **27b**: δ 3.68 (2 H, d, *J* 6 Hz), 4.42 (H-6, s), 4.73 (1 H, d, *J* 6 Hz), 5.09 (1 H, d, *J* 9 Hz), 5.92 (1 H, d, *J* 9 Hz), 6.48 (H-4, s), 6.9 and 7.2 (4 H, Ar).

Sodium 5-chloro-1-[4-(2,3-diacetoxypropoxy)phenacyl]-2-oxo-1,2,3,4-tetrahydropyrimidine-4-sulfonate (**28a**). Sodium pyrosulfite (0.45 g, 2.4 mmol) was dissolved in water (10 ml) and the pH adjusted to ca. 7 with 1 M sodium hydroxide. 5-Chloro-1-[4-(2,3-diacetoxypropoxy)phenacyl]-2(1*H*)pyrimidinone (0.5 g, 4.7 mmol) was added and the reaction stirred at ambient temperature for 24 h. The pyrimidinone slowly dissolved and the product precipitated towards the end of the reaction; yield 1.7 g (69 %). ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.00 (OAc), 2.02 (OAc), 4.2–4.4 [5 H, m, $CH_2CH(OAc)CH_2OAc$ and H-4], 4.47 and 5.10 (2 H, AB, *J* 18 Hz, NCH_2), 5.3 (CH, m), 6.56 (H-6, s), 7.2 and 7.9 (4 H, Ar), 7.18 (NH, d, *J* 3 Hz).

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