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Researches on Antiviral Agents. 41. Studies on the Chemistry of 6-Methyl-2-methoxy-4-O-acyloxy and 6-Methyl-2,4-di-O-acyloxypyrimidine Derivatives as New Acylation Reagents and Inhibitors of Uracil DNA Glycosylases.

Maurizio Botta[a]*, Raffaele Saladino[b], Gabriella Gentile[c], Vincenzo Summa[c], Rosario Nicoletti[c]*, Annalisa Verri[d], Federico Focher[d], Silvio Spadari[d].

[a] Dipartimento Farm. Chim. Tecn., Università di Siena, Via Banchi di Sotto 55, 53100 Siena. [b] Dipartimento AgroChimico AgroBiologico, Università degli Studi di Viterbo "La Tuscia", Via San Camillo de Lellis, 01100 Viterbo, Italy. [c] Dipartimento di Chimica, Università degli Studi di Roma "La Sapienza", p.le Aldo Moro 5, 00185 Roma, Italy. [d] C.N.R, Istituto di Genetica Biochimica ed Evoluzionistica, Via Abbiategrasso 207, 27100 Pavia, Italy.

Abstract: The synthesis of 6-methyl-2-methoxy-4-O-acyloxy and 6-methyl-2,4-di-O-acyloxypyrimidine derivatives **2** and **5** along with their properties as acylating agents of amines, alcohols, thiols and α-amino acids have been reported. Interestingly some of the title products revealed an inhibitory activity against the human and herpetic DNA glycosylases.

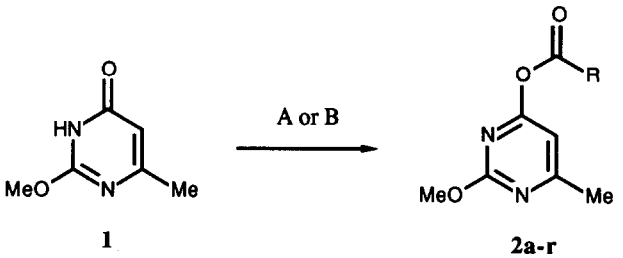
The problem of the regioselective (N versus O) acylation and alkylation of uracil and its derivatives has be investigated for a long time by chemists with the aim of synthesizing natural nucleic acids[2]. For this reason a lot of effort has been devoted to the study of these reactions on uracil and thymine while little effort has been devoted to the acylation of 6-substituted uracils. Since we have been involved in the last few years in the synthesis[3] and in the biological evaluation[4] of 6-substituted uracils, we became interested in studying this problem. It is worth pointing out, in fact, that the latter series of compounds shows a completely different regioselectivity in glucosylation reactions[5]. This particular behaviour has been justified by the authors with the encumbering of the C-6 substituents during electrophilic attack at the N-1 position of the uracil ring.
We communicated in a previous letter[6] a general synthesis of 6-methyl-2-methoxy-4-acyloxypyrimidines **2a-c** through a regiospecific acylation of the C-4 carbonyl of 6-methyl-2-methoxy-4(3H)-pyrimidinone **1** with acyl chlorides in pyridine-acetonitrile (solvent). This result disagrees with the data reported by Reese on the acylation of uracil and thymine[7] where only the 1,3-diacylated derivatives are generally observed. An analogous result was also reported by Novacek when uracil was heated under reflux in a pyridine-dioxane solvent mixture with a large excess of benzoyl chloride[8]. We are reporting here a full account of the synthesis and the reactivity of products **2a-c** along with the synthesis and the reactivity of other acylated products **2d-r** and **5**.

Acylation of 6-methyl-2-methoxy-4(3H)-pyrimidinone **1** with several acyl chlorides (method A, scheme 1) gave the 4-O-acyl-derivatives **2a-c** in very good yields (see table 1). Since the use of acyl chlorides could be a

limiting step, for example, when this procedure is applied to the synthesis of protected amino acids, many different condensing agents were tried for the direct condensation of **1** with acids.

Finally, compound **1** was reacted with several aromatic acids in THF in the presence of EDCI (method B, scheme 1) to afford products **2d-r** in good yields.

Scheme 1



1 **2a-r**

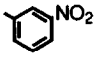
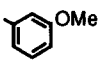
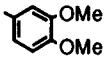
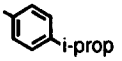
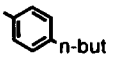
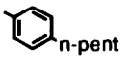
Method A: RCOCl , MeCN / Pyridine, r.t.
Method B: RCO_2H , EDCI, DMAP, THF.

All products are crystalline compounds and their structures were proved by their spectroscopic properties (NMR downfield shift of the C-5 proton resonance of **2** with respect to the same proton in **1** and the presence in the IR spectrum of only one absorption of the carbonyl moiety). Compounds **2a-r** are stable at room temperature, and can be stored in the refrigerator for a long period of time. To extend this procedure, N-protected amino acids and aliphatic acids were used, but the reaction did not succeed, even under different experimental conditions.

All products **2a-r** were tested as antimicrobial and antiviral agents[9], but no compound showed better antimicrobial activity than that described for **2c**[6] while compounds **2i** and **2n-p** showed interesting antiviral activity as DNA-glycosylase inhibitors (vide infra). Furthermore compounds **2** showed interesting N-acylating properties: thus, as a general procedure, by reacting **2c** with the appropriate amines, quantitative yields of amides **3a-d** were obtained in periods ranging from a few minutes to 24 h (scheme 2, table 2).

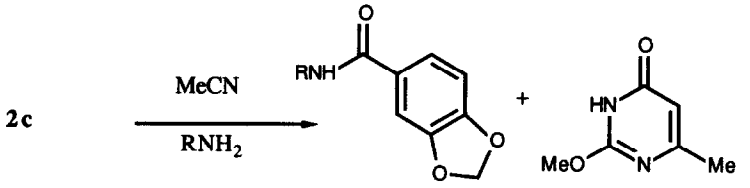
Reactions were carried out by simply dissolving **2c** and the amine in MeCN; and at the end of the reaction, dilution with ethyl ether caused almost complete precipitation of pyrimidinone **1**. Removal of the solid, washings with 2N aqueous HCl, drying and evaporation of the solvents gave crystalline products (table 2).

Table 1.REACTION OF 6-METHYL-2-METHOXY-4(3H)-PIRIMIDINONE 1 WITH ACYLCHLORIDES AND AROMATIC ACIDS

METHOD	COMPOUNDS	R	REFLUX TIME	YIELD(%)	REACTION TIME at 25°C (h)
A	**2a**	Ph	0	95	24
A	**2b**	Me	0	78	24
A	**2c**		0	98	24
B	**2a**	Ph	0	73	24
B	**2d**		0	80	16
B	**2e**		0	81	18
B	**2f**		5	27	24
B	**2g**		2	33	24
B	**2h**		8	13	24
B	**2i**		0	79	18
B	**2j**		5	48	18
B	**2k**		2	48	24
B	**2c**		0	54	18
B	**2l**		0	56	20
B	**2m**		2	79	18
B	**2n**		2	75	18
B	**2o**		2	71	18

METHOD	COMPOUNDS	R	REFLUX TIME	YIELD(%)	REACTION TIME at 25 °C (h)
B	2p		0	83	18
B	2q		0	90	18
B	2r		0	73	18

Scheme 2



3a-d **1**

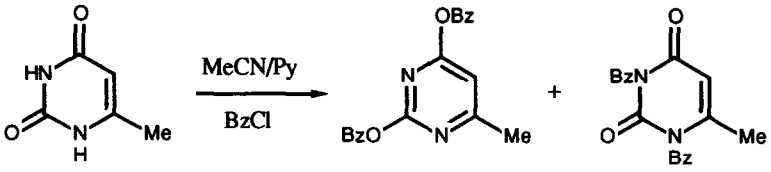
Table 2.REACTION OF 6-METHYL-2-METHOXY-4-O-PIPERONYLPYRIMIDINE 2c WITH AMINES

PRODUCTS	R	YIELD(%)
3a	cyclohexyl	95
3b	Ph	98
3c	CH_2CH_2OH	94
3d	p-C_6H_4OH	90

It is interesting to note that in the case of aminoethanol and p-aminophenol only the N-acylated products were obtained (**3c** and **3d**). Structures of products **3a-d** were confirmed by IR, NMR and mass spectra. No reaction occured between **2c** and alcohols at room temperature and at a higher temperature primary alcohols (e.g. n-BuOH) gave the corresponding esters in poor yields. However, when DMAP was added to the reaction mixtures, the alcohols were easily acylated. Ethanol and cholesterol reacted with **2a** and yielded the corresponding acyl derivatives at room temperature in nearly quantitative yields. α-Amino acids did not react with our reagent even in the presence of dimethylaminopyridine (DMAP).

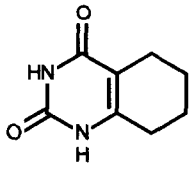
Heterocyclic reagents with acylating properties have been extensively used in the synthesis of N-protected amino acids, nevertheless little has been reported on pyrimidine derivatives. In order to increase the acylating properties of reagent **2** we turned our attention to the possibility of obtaining 2,4-diacyloxypirimidines. Compound **4** was reacted with benzoyl chloride in pyridine-MeCN at 25°C to afford the di-acyloxy derivative **5** as the main product (51%), and the 1,3-dibenzoylated isomer **6** (7%) as a byproduct (scheme 3).

Scheme 3

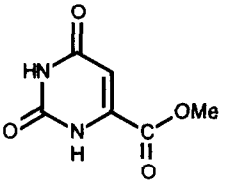


4 **5** **6**

These data compared to that reported by Reese[7] and Novacek[8] show that the regioselectivity (O versus N) of this acylation is determined by the presence of a substituent at C-6 of the uracil ring. To better understand the influence of the C-6 substituent on the regioselectivity of the acylation we reacted compounds **7**[3] and **8** with benzoyl chloride.



7



8

In accordance with data described for **1**, a great prevalence of the 2,4-disubstituted derivative was obtained in the case of compound **7**, while in the case of compound **8** a ratio of 3 to 1 of the 1,3-disubstituted product to the 2,4-disubstituted one was obtained. Results clearly indicate that steric hindrance at the N-1 position exerted by the C-6 group is not the only important factor influencing the regioselection of the reaction, as previously described for the glucosylation of 6-methyl uracil[5] indeed electronic effects play a role; the experiment clearly indicate that an EDG at C-6 gives rise to O-acylated products, while an EWG at C-6 favors N-acylation.

These results can best be explained by the fact that an EDG at C-6, through resonance, will increase the nucleophylicity of the conjugated carbonyl oxygen giving rise to O-acylated products. The EWG at C-6,

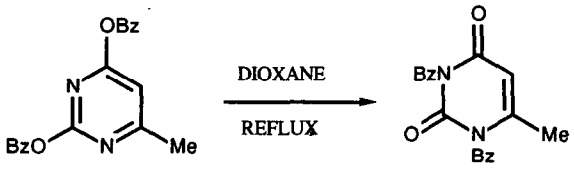
however, will destabilize the conjugated carbonyl system thereby lowering the reactivity of the oxygen at C-4, hence favouring N-acylation.

Furthermore, Kamimura has shown[10], by ^{13}C NMR studies, that in N-benzylated uracils the C-6 atom is more electron-rich than in the corresponding oxygen derivatives. This adds weight to our reasoning above since EWG's would stabilize the more electron-rich C-6 atom of the N-acylated products (and EDG's would destabilize less the less electron-rich C-6 atom of O-acylated products).

To evaluate the generality of the C-6 substituent effect in the acylation reaction we repeated the benzoylation of **4** by refluxing the substrate with benzoyl chloride-pyridine (5/2 v:v) in dioxane (Novacek's-procedure[8]) and only the 1,3-dibenzoyl derivative **6** was obtained.

Ulbricht reported[11] that the 2,4-diglucopyranosyl uracil, obtained as the kinetic product from the reaction of the silver salt of uracil with 2',3',4',6'-tetra-O-acetylglucopyranosyl bromide, was transformed into the 1,3-bisglucoside of uracil, the thermodynamic product, by refluxing the former isomer in toluene in the presence of mercuric bromide. No records are available in the literature reporting a similar rearrangement of 2,4-diacylated pyrimidinones. Consequently we submitted **5** to refluxing dioxane and product **6** was quantitatively obtained.

Scheme 4



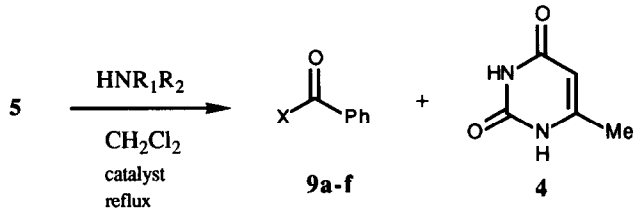
5 **6**

The 1,3-derivative **6** seems to be in this case, the thermodynamic isomer while **5** the kinetic one; in fact, was also obtained by us directly from **4** following Novacek's procedure[8], i.e. in refluxing dioxane. O-Acylation of 6-methyl uracil with acyl chloride followed by refluxing in dioxane is actually a very good way of obtaining 6-methyl-1,3-dibenzoyl uracil **6**, and yields are in fact higher than that obtained by the direct benzoylation of 6-methyl uracil using Novacek's procedure; in fact, in our hands, the work up of the direct method was very low yielding. Subsequently, to establish whether the reaction proceeded with an intramolecular rearrangement of the benzoyl moiety or an intermolecular acylation of a new molecule of substrate, we refluxed a 1:1 mixture of compound 5 and 6-ethyl uracil in dioxane. 1,3-Dibenzoylated uracil **6** was obtained in quantitative yield while 6-ethyl uracil was recovered unchanged, showing that the reaction proceeds with a never before described intramolecular rearrangement of the benzoyl moiety. It is interesting to note that when the same rearrangement was tried on **2a** it did not afford the N-acylated derivative.

The acylation of 6-methyl uracil was also carried out using acetyl chloride and piperonyl chloride as acylating reagents. The reaction proceeded only in the case of piperonyl chloride giving the corresponding 2,4-diacylated product and shows that the aromaticity of the acylating reagents, as we have previously observed in the case of the acylation of compound **1** with 1,1-(3-dimethylaminopropil)-3-ethyl-carbodiimide (EDCI), is an

important feature of this reaction. Finally with the synthesis of substrate **5** in hand, we evaluated its acylating properties (scheme 5).

Scheme 5



When **5** was reacted with diethylamine in a 0,5 to 1 molar ratio the reaction proceeded at room temperature affording the corresponding amide **9c** in approximately 50% yield and went to completion only after refluxing for 12 hours. Subsequently, for making the reaction faster, compound **5** was refluxed with diethylamine in the presence of a catalytic amount of triethylamine, and the amide **9c** was obtained in good yield (92%) in two hours.This procedure was used for the acylation of several amines and the corresponding amides **9a-d** have been obtained in very good yields and in very mild conditions (scheme 5,table 3).

Table 3.ACYLATION OF AMINE GROUP

COMPOUNDS	R_1	R_2	YIELD(%)	CATALYST	TIME
9a	Bu	H	64	Et_3N	15 min
9b	Me	allyl	90	Et_3N	1 h
9c	Et	Et	92	Et_3N	2 h
9d	C_6H_5	H	57	DMAP	48 h
9e	$OH(CH_2)_2$-	H	99	none	15 min
9f	$OH(CH_2)_4$-	H	64	none	15 min
10a	CO_2-tBu-CH_2-	H	98	none	30 min
10b	CO_2-Me-Ala	H	93	DMAP	30 min
10c	CO_2-Me-Val	H	86	DMAP	35 min

Products **9a-d** were easily obtained after filtration of **4** (produced during the reaction) and solvent evaporation. Only in the case of **9d** a chromatographic purification was necessary.

When compound **5** was reacted with amino alcohols in refluxing CH_2Cl_2 regiospecific acylation of N

occurred **9e-f**. Acids did not react while amino acids (protected at the acid function to obviate solubility problems) reacted very rapidly and in good yields to give the corresponding amides **10a-c** (table 3). Alcohols, phenols and thiophenols also reacted with **5** in the presence of catalytic amounts of Et_3N. Better yields and faster reaction times have been subsequently obtained using DMAP (table 4, table 5, scheme 6).

Scheme 6

Table 4.EFFECT OF CATALYST ON THE ACYLATION OF ALCOHOLS.

R	X	CATALYST	SOLV/T°C	YIELD/TIME
Et	O	MeONa	EtOH/60	33% ; 24 h
Et	O	Et_3N	EtOH/60	18% ; 24 h
Et	O	DMAP	EtOH/60	47% ; 24 h

All the acylated products **9**, **10**, **11** have been identified through their spectroscopic data and by comparison with samples prepared by a different route.

Table 5.ACYLATION OF ALCOHOLS AND THIOLS

COMPOUNDS	X	R	YIELD(%)	TIME
11a	O	Et	47	24 h
11b	O	Bu	88	2 h
11c	O	CH_2Ph	81	15 min
11e	S	C_6H_5	84	24 h

From these data we can assume that **5** shows some advantages over 6-methyl-2-methoxy-4-acyloxypyrimidine **2.** First of all due to the stoichiometry of the reagent, half the amount of **5** can be used for the same amount of substrate which is to be acylated even if more drastic reaction conditions are required. Uracil, in the end of the reaction, precipitates and can be completely removed by filtration in both cases. Acylation is faster when **5** is used (in 2:1 ratio) and, in addition, amino acids can also be acylated by **5**. Further work in this area is in progress in our laboratories.

Biological Part

Uracil, a base normally confined to RNA, can occur in DNA as a consequence of cytosine deamination and misincorporation, in place of thymine, by DNA polymerases during DNA synthesis[12]. Normally uracil is removed from DNA by action of uracil DNA-glycosylase, which catalyzes the specific removal of uracil by cleaving the N-glycosidic bond linking the base to the deoxyribose phosphate backbone. This enzyme is present in viruses, prokaryotic and eucaryotic cells[13,14]. Incorporation of a small amount of uracil into DNA during DNA replication has been observed in some viruses, including polyoma virus[15] and adenovirus[16], in bacteria[17] and in human lymphocytes in culture[18].

Table 6. EFFECT OF COMPOUNDS 2a-t ON THE HUMAN AND HSV-1 URACIL-DNA GLYCOSYLASES.

	IC50(μm)[a]	
COMPOUNDS	HUMAN UDG	HSV UDG
2a	>500	>500
2b	>500	>500
2c	>500	>500
2d	>500	>500
2e	>500	>500
2f	>500	>500
2g	>500	200
2h	>500	>500
2i	80	50
2j	>500	>500
2k	>500	>500
2l	>500	>500
2m	>500	>500
2n	22	4
2o	23	22
2p	57	60
2q	>500	>500
2r	>500	>500

a: Concentration causing 50% inhibition of uracil-DNA glycosylase (UDG) activity.

It was also observed in lymphocytes that increased levels of dUTP, due to the blocking of the thymidylate synthetase by methotrexate, favour the misincorporation of uracil into DNA[18] leading to an increased level of mutations in DNA. In the case of Herpes simplex(HSV) infections, we have recently proposed that the viral encoded uracil-DNA glycosylase, non-essential for proliferation of HSV1 in cell cultures, could play a key role in nerve cells for "cleaving" of the viral genome during the reactivation from latency in nerve cells[18]. A selective inhibitor of the herpetic uracil-DNA glycosylase would thus be useful in preventing viral reactivation. Thus we have studied the effect on human and HSV1 uracil-DNA glycosylases of compounds **2a-r.**

The screening of these uracil derivatives against the purified human HeLa and HSV1 uracil-DNA glycosylase revealed an interesting inhibitory activity, in the μM range, against both enzymes by compounds **2i** and **2n-p** (see figure 1 and table 6).These data show that a long aliphatic chain substituent in the aryl moiety is an essential feature for the inhibition properties of compounds **2i** and **2n-p**. It is reasonable to suggest that the

long aliphatic chain is important in the transport of molecules through the lipophilic cell membrane. When used in the presence of drugs which lead to an increased level of dUTP, such as methotrexate or inhibitors of dUTPase, they would thus limit the removal of uracil from DNA with a consequently higher frequency of mutagenesis. This could preferentially occur in rapidly proliferating neoplastic cells where such inhibitors might thus exert some selective cytotoxic effect. Attempts are in progress to obtain other selective inibitors of the herpetic enzyme[20] which may prevent the viral reactivation from latency in nerve cells.

Experimental Part

Melting points were determined with a Kofler hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 298 spectrophotometer. ^1H and ^{13}C NMR spectra were determined for solutions in $CDCl_3$ (unless specified otherwise) on Varian Gemini 200 or Varian XL 300 instruments. Mass spectra were obtained on a Kratos MS 80 spectrometer. Flash chromatography was performed using Kiesegel 60 (230-400 mesh, E. Merck). All the reagents were purchased from the Aldrich Chemical Co., Inc.

Preparation of 2-methoxy-6-methyl-4-O-acyl-pyrimidinone derivatives 2.

(Method A)

In a 50 ml flask, equipped with a magnetic stirring bar, 6-methyl-2-methoxy-4(3H)-pyrimidinone 1 (1 mmol) was dissolved in a 5:1 v/v acetonitrile-pyridine solution (10 ml). The appropriate acyl chloride (1.2 mmol) was then added and the reaction mixture was stirred at room temperature for 3h. The reaction mixture was evaporated, dissoved in EtOAc (50 ml), washed with HCl (2 N), water and brine. Evaporation of the dried solvent (Na_2SO_4) and purification by column chromatography ($CHCl_3/CH_3OH$= 99:1) gave pure **2a-c**.

2a: mp 137-139 °C; IR ($CHCl_3$, cm^{-1}) 1750, 1610; ^1H-NMR ($CDCl_3$): 2.51 (3H, s, CH_3), 3.87 (3H, s, OCH_3), 6.67 (1H, s, CH), 6.91-7.76 (5H, m, CH); MS m/e 244 (M^+).

2b: mp 121-122 °C; IR ($CHCl_3$, cm^{-1}) 1750, 1605; ^1H-NMR ($CDCl_3$): 2.32 (3H, s, CH_3), 2.45 (3H, s, CH_3), 3.95 (3H, s, CH_3), 6.61 (1H, s, CH); Anal. Calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.69; H, 5.50; N, 15.40. MS m/e 182 (M^+).

2c: mp 119-120 °C; IR ($CHCl_3$, cm^{-1}) 1750, 1605; ^1H-NMR ($CDCl_3$): 2.51 (3H, s, CH_3), 3.92 (3H, s, CH_3), 6.70-7.80 (1H, s, CH), 6.70-7.80 (3H, m, CH); Anal. Calcd. for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.27; H, 4.22; N, 9.80. MS m/e 288 (M^+).

(Method B)

In a 50 mL flask, equipped with a magnetic stirring bar, acid (2 mmol.), EDCI (2 mmol.) and 6-methyl-2-

methoxy-4(3H)-pyrimidinone **1** (1 mmol.) were dissolved in THF (20 mL). A catalytic amount of DMAP (dimethylaminopyridine) was then added to this mixture. Reaction times and temperatures depended on the acid used (see table 1). The reaction was followed by TLC ($CHCl_3/CH_3OH$). After the reaction was complete the solvent was evaporated and the residue dissolved in $CHCl_3$. The solution was washed with water and with brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo.The residue was purified on

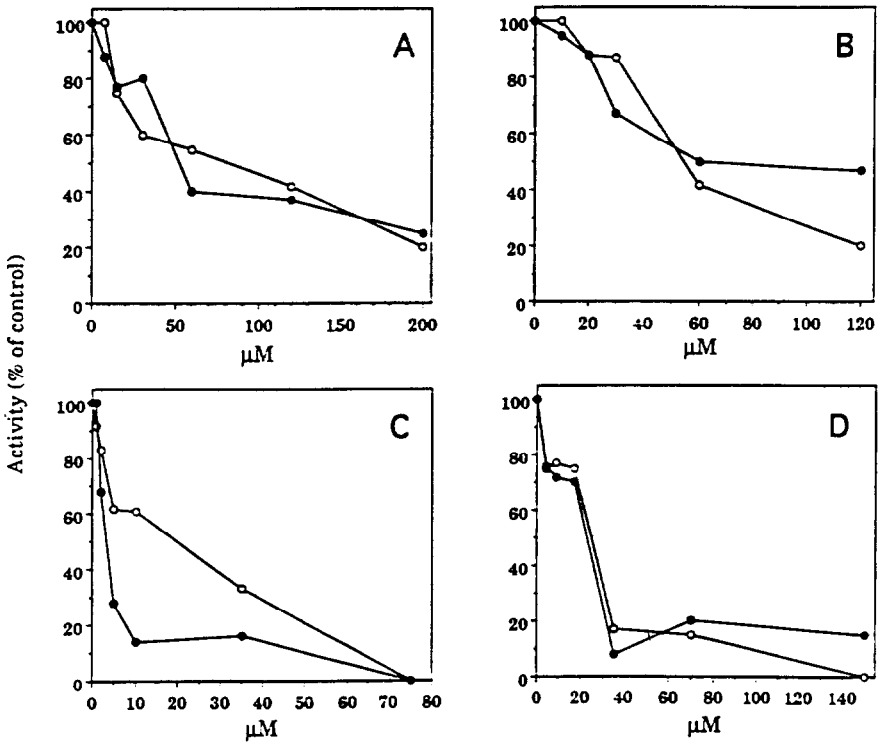


Fig. 1. Dose-response curves for uracil-DNA glycosylase inhibition by compounds 2i and 2n-p.
The HSV (o) and human (o) enzymes were assayed as described in the Experimental Part with the addition of stock compounds dissolved in DMSO. Control activity correspond to enzyme activity in the presence of an identical concentration of the solvent. A: 2i: B: 2p: C: 2n and D: 2o

preparative TLC affording products **2a** and **2d-r**; yields are reported in table 3.

2d: mp 81-82 °C; IR ($CHCl_3$, cm^{-1}) 1740, 1600; ^{1}H-NMR ($CDCl_3$): 2.50 (3H, s, CH_3), 3.81 (3H, s,

OCH_3), 4.0 (3H, s, OCH_3), 6.71 (1H, s, CH), 7.70-8.82 (4H, m, CH); Anal. Calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.27; H, 5.18; N, 10.25. MS m/e 274 (M^+).

2e: mp 54-55 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1605; ^{1}H-NMR ($CDCl_3$): 2.33 (3H, s, CH_3) , 2.40 (3H, s, CH_3), 3.90 (3H, s, OCH_3), 6.71 (1H, s, CH), 7.20-8.0 (4H, m, CH); Anal. Calcd. for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.27; H, 5.45; N, 10.83. MS m/e 259 (M^+).

2f: mp 116-117 °C; IR ($CHCl_3$, cm^{-1}) 1750, 1610.; ^{1}H-NMR ($CDCl_3$): 2.49 (3H, s, CH_3) , 3.98 (3H, s, OCH_3), 6.75 (1H, s, CH),7.45-8.12 (4H, m, CH); Anal. Calcd. for $C_{13}H_{11}ClN_2O_3$: C, 56.03; H, 3.98; N, 10.05. Found: C, 56.10; H, 4.00; N, 10.15. MS m/e 279 (M^+).

2g: mp 102-103 °C; IR ($CHCl_3$, cm^{-1}) 1740, 1605; ^{1}H-NMR ($CDCl_3$): 2.47 (3H, s, CH_3), 3.97 (3H, s, OCH_3), 6.73 (1H, s, CH), 7.10-8.05 (4H, m, CH); Anal. Calcd. for $C_{13}H_{11}IN_2O_3$: C, 42.18; H, 3.00; N, 7.57. Found: C, 42.25; H, 3.10; N, 7.59. MS m/e 370 (M^+).

2h: mp 96-98 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1600; ^{1}H-NMR ($CDCl_3$): 2.49 (3H, s, CH_3), 3.99 (3H, s, OCH_3), 6.73 (1H, s, CH), 7.17-8.23 (4H, m, CH); Anal. Calcd. for $C_{13}H_{11}N_2O_3$: C, 59.54; H, 4.23; N, 10.68. Found: C, 59.60; H, 4.28; N, 10.70. MS m/e 262 (M^+).

2i: oil ; IR ($CHCl_3$, cm^{-1}) 1740, 1620; ^{1}H-NMR ($CDCl_3$): 0.79 (3H, m, CH_3), 1.48 (2H, m, CH_2), 2.28 (3H, s, CH_3), 2.42 (2H, m, CH_2), 3.85 (3H, s, OCH_3), 6.72 (1H, s, CH), 7.25-8.15 (4H, m, CH); MS m/e 286 (M^+).

2j: mp 93-94 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1615; ^{1}H-NMR ($CDCl_3$): 2.40 (3H, s, CH_3), 3.80 (3H, s, OCH_3), 6.61 (1H, s, CH), 7.51-8.22 (4H, m, CH); Anal. Calcd. for $C_{13}H_{11}N_3O_5$: C, 53.98; H, 3.83; N, 14.53. Found: C, 54.05; H, 3.93; N, 14.60. MS m/e 289 (M^+).

2k: mp 102-103 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1605; ^{1}H-NMR ($CDCl_3$): 2.42 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 6.69 (1H, s, CH), 7.29-7.80 (4H, m, CH); Anal. Calcd. for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.37; H, 5.19; N, 10.27. MS m/e 274 (M^+).

2l: mp 106-107 °C; IR ($CHCl_3$, cm^{-1}) 1740, 1600; ^{1}H-NMR ($CDCl_3$): 2.31 (3H, s, CH_3), 3.73 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.61 (1H, s, CH), 6.75-7.67 (3H, m, CH); Anal. Calcd. for $C_{15}H_{16}N_2O_5$: C, 59.21; H, 5.30; N, 9.21. Found: C, 59.30; H, 5.38; N, 9.25. MS m/e 304 (M^+).

2m: oil; IR ($CHCl_3$, cm^{-1}) 1740, 1605; ^{1}H-NMR ($CDCl_3$): 1,30 (6H, m, CH_3), 2.49 (3H, s, CH_3), 2.90 (1H, m, CH), 4.05 (3H, s, OCH_3), 6.75 (1H, s, CH), 7.25-8.15 (4H, m, CH); MS m/e 286 (M^+).

2n: oil; IR ($CHCl_3$, cm^{-1}) 1750, 1610; ^{1}H-NMR ($CDCl_3$): 0,90 (3H, m, CH_3), 1.0-1.95 (4H, m, CH_2), 2.48 (3H, s, CH_3), 2.80 (2H, m, CH_2), 4,05 (3H, s, OCH_3), 6.81 (1H, s, CH), 7.30-8.25 (4H, m, CH); MS m/e 300 (M^+).

2o: oil; IR ($CHCl_3$, cm^{-1}) 1740, 1600; ^{1}H-NMR ($CDCl_3$): 0,75 (3H, m, CH_3), 0.89-1.71 (6H, m, CH_2), 2.35 (3H, s, CH_3), 2.65 (2H, m, CH_2), 3.95 (3H, s, OCH_3), 6.65 (1H, s, CH), 7.10-8.05 (4H, m, CH); MS m/e 314 (M^+).

2p: oil; IR ($CHCl_3$, cm^{-1}) 1740, 1610; ^{1}H-NMR ($CDCl_3$): 2.23 (9H, m, CH_3), 3.82 (3H, s, OCH_3), 6.63 (1H, s, CH), 7.11-7.98 (3H, m, CH); MS m/e 272 (M^+).

2q: mp 71-72 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1618; ^{1}H-NMR ($CDCl_3$): 2.20 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 6.57 (1H, s, CH), 6.90-7.70 (3H, m, CH); Anal. Calcd. for $C_{11}H_{10}N_2O_3S$: C, 52.79; H, 4.03; N, 11.19. Found: C, 52.69; H, 4.08; N, 11.25. MS m/e 250 (M^+).

2r: mp 89-90 °C; IR ($CHCl_3$, cm^{-1}) 1735, 1600; ^{1}H-NMR ($CDCl_3$): 2.50 (3H, s, CH_3), 4.0 (3H, s, OCH_3), 6.81 (1H, s, CH), 6.30-7.81 (3H, m, CH); Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.41; H, 4.30; N,

11.96. Found: C, 56.53; H, 4.35; N, 12.00. MS m/e 234 (M^+).

Benzoylation of 6-methyluracil 4.

In a 250 mL flask, equipped with a magnetic stirring bar, 6-methyluracil **4** (2.80 g, 22 mmol.) was dissolved in CH_3CN (60 mL) in the presence of benzoylchloride (77 mmol.) and dry pyridine (149 mmol.). The mixture was stirred at room temperature for 16 h and followed by TLC ($CHCl_3/CH_3OH$=9.8/0.2). After the reaction was completed the mixture was evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed with HCl (2N), aqueous $NaHCO_3$ and brine, then dried and evaporated to dryness. The residue was purified by flash chromatography on silica gel (n-hexane/AcOEt) affording 2,4-dibenzoyl-6-methyl-uracil **5** and 1,3-dibenzoyl-6-methyluracil **6**:

5: (3.7 g, 51%), mp 69-70 °C; IR ($CHCl_3$, cm^{-1}) 1745, 1645, 1580, 1550, 1430, 1080; ^{1}H-NMR ($CDCl_3$): 2.61 (3H, s, CH_3), 7.20 (1H, s, CH), 7.48 (5H, m, CH), 8.16 (5H, m, CH); ^{13}C NMR ($CDCl_3$) : 24.04 (CH_3), 110.61 (CH), 128.04 (C), 128.46 (C), 128.71 (CH), 128.84 (CH), 130.65(CH), 134.24 (CH), 134.69 (CH), 161.29 (C), 163.29 (C), 164.12 (C), 167.22 (C), 173.82 (C); Anal. Calcd. for $C_{19}H_{14}N_2O_4$: C, 68.26; H, 4.22; N, 8.38. Found: C, 68.17; H, 4.18; N, 8.25. MS m/e 334 (M^+).

6: (0.5 g, 7%), mp 135-137 °C; IR ($CHCl_3$, cm^{-1}): 1750, 1730, 1670, 1620, 1400; ^{1}H-NMR ($CDCl_3$): 2.12 (3H, s, CH_3), 5.77 (1H, s, CH), 7.55 (6H, m, CH), 7.90 (4H, m, CH); ^{13}C NMR ($CDCl_3$): 18.79 (CH_3), 102.42 (CH), 129.51 (CH), 130.07 (CH), 130.34 (CH), 131.28 (C), 131.56 (C), 135.31 (CH), 135.79 (CH), 149.26 (C), 151.13 (C), 161.47 (C), 168.08 (C), 168.44 (C); Anal. Calcd. for $C_{19}H_{14}N_2O_4$: C, 68.26; H, 4.22; N, 8.38. Found: C, 68.19; H, 4.20; N, 8.35. MS m/e 334 (M^+). MS m/e 334 (M^+).

Synthesis of 1,3-dibenzoyl-6-methyluracil 6.

2,4-Dibenzoyl-6-methyl-uracil **5** (0.42 mmol.) and freshly distilled dry dioxane (5 ml) were placed in a 25 ml flask, equipped with a condenser and a drying tube. The stirred solution was then refluxed. The reaction was followed by TLC (hexane/AcOEt= 6/4) and after 4h the initial product was completely transformed into the title compound. The mixture then was evaporated to dryness affording the 1,3-dibenzoyl-6-methyluracil **6**: (58 mg, 82%).

General Procedure for the Acylation of amines and alcohols using 2c as acylating agent.

Compound **2c** (1 mmol.) was dissolved in CH_3CN (5 ml) and the amine (1 mmol.) was added. The mixture was stirred at room temperature until **2c** was not detectable. Dilution with Et_2O followed by filtration, washing with 2N HCl and evaporation to dryness, gave products **3a-d** in yields reported in table 3.

In the case of **3e-f**, DMAP (20 mg) was added. All products showed analytical data in accordance with the literature.

General Procedure for the Acylation of amines, alcohols, thiols and α-amino acids using 5 as acylating agent.

In a typical experiment 2,4-dibenzoyl-6-methyl-uracil **5** (150 mg, 0.45 mmol.) was dissolved in dry CH_2Cl_2 (10 mL) in a 25 mL flask, equipped with a reflux condenser and a magnetic stirring bar. Amine (0.90 mmol.) and a catalytic amount of DMAP or other catalyst (see table 4 and 5) were added and the mixture was refluxed with stirring. After the reaction was completed, the precipitated 6-methyl-uracil was filtered off and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane-AcOEt or $CHCl_3$-CH_3OH) affording the products **9a-f**, **10a**, **11a-e**. All the products have ^1H NMR, ^{13}C NMR and elemental analyses in accordance with those reported in literature.

Biological methods

Purification of human uracil-DNA glycosylase.

HSV1 infection, harvesting of infected cells and purification of HSV1 uracil-DNA glycosilase were performed as described by Focher et al.[19,20]. Human uracil-DNA glycosylase was purified from the cytoplasm of HeLa cells by a procedure partially derived from that of Krokan and Wittwer[21] and described by Focher et al.[20].

Uracil-DNA glycosylase assay

Assays in a final volume of 25 µl each contained 100 mM Tris-HCl (pH 8.0), 5 mM DDt (dithiothreitol), 10 mM EDTA, 500 ng of (3H)d-UMP-labelled DNA (40 cpm/ng; 220 cpm/pmol uracil; 3.6 µM uracil) prepared as described[22], 4 µg of unlabelled activated DNA and the enzyme (0.3 units) to be tested (when HSV1 enzyme was used, BSA was added to the test tube in order to obtain a protein concentration comparable with that of the human enzyme). After the incubation at 37°C for 30 min, 20 µl of the mixture spotted on GF/C filters (Whatman). The filters were washed three times in 5% (v/v) trichloroacetic acid for 5-10 min and twice in ethanol. The filters were dried and the acid-insoluble radioactivity was measured by scintillation counting in 1 ml of scintillation fluid. One unit of uracil-DNA glycosydase removes 1 nmol of uracil from DNA in 1h. at 37°C.

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