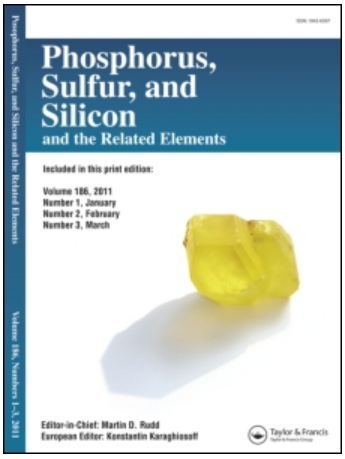

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THIO ANALOGS OF PYRIMIDINE BASES: SYNTHESES AND EIMS STUDIES OF NEW 2-(AND 4-) O-(*M*- AND *P*-) HALOBENZYLTHIO-6-METHYLURACILS

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THIO ANALOGS OF PYRIMIDINE BASES: SYNTHESES AND EIMS STUDIES OF NEW 2-(AND 4-) *O*-(*M*- AND *P*-) HALOBENZYLTHIO-6-METHYLURACILS

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Twelve new *ortho*-, *meta*- and *para*- chloro-(and bromo)-substituted derivatives of 2- and 4-benzylthio-6-methyluracils have been prepared in the reactions of 2- and 4-thio-6-methyluracil with the corresponding benzyl halides. EI induced mass spectral fragmentation of these compounds was investigated. Fragmentation pathways are proposed on the basis of accurate mass and metastable transitions measurements. The correlation between the abundance of the $M^{+\cdot}$ and the selected fragment ions of the investigated compounds is discussed. The data obtained create the basis for distinguishing structural isomers.

Keywords: 2- and 4-benzylthio-6-methyluracils; *ortho*-(*meta*- and *para*-)halobenzyl halides; EIMS; structural isomers.

INTRODUCTION

Thio derivatives of pyrimidine bases are of interest because of their biological and pharmacological activities e.g. as minor components of t-RNA or as anticancer and antithyroidal drugs as well as sedatives.[1–7] Owing to the biological importance of modified thio analogues of pyrimidine bases, interest in their spectral analysis is currently very active. Mass spectrometry continues to be the most convenient and effective method for the determination of the nature of covalent modifications to thio analogues of nucleobases.[8–11] Our previous work

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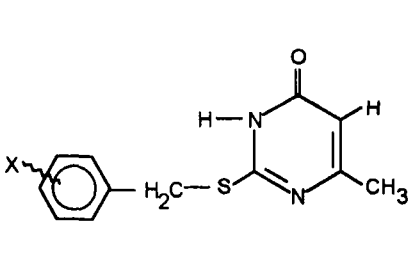
dealing with the synthesis of S-substituted derivatives of thiouracils[12] and their EIMS studies[13,14] enabled us to introduce *ortho-* (*meta-* and *para-*)halobenzyl substituents on the sulfur atom of the 6-methylthiouracils. In this paper we describe the synthesis and characteristics of 12 new 2- and 4-[*ortho-* (*meta-* and *para-*) chloro- (and bromo-)benzylthio-]-6-methyluracils (**1–12**) (Fig. 1).

RESULTS AND DISCUSSION

Scheme 1 illustrates the synthetic approach chosen for the preparation of 2-benzylthio-6-methyluracils (**1–6**) and 4-benzylthio-6-methyluracils(**7–12**). The 2- and 4-thio-6-methyluracils were selected as the starting materials, along with the *ortho-* (*meta-* and *para-*) chlorobenzyl chlorides, as well as *ortho-* (*meta-* and *para-*)bromobenzyl bromides. The reactions of these compounds were carried out in 0.15 (**4**, **11**) or 0.2 (**7**, **8**, **9**) as well 0.3 (**1–3**, **5**, **6**, **11**, **12**) N NaOH methanolic solutions at room temperature. Twelve new 2-benzylthio-6-methyluracils (**1–6**) as well as 4-benzylthio-6-methyluracils (**7–12**) were obtained in these reactions of nucleophylic substitution.



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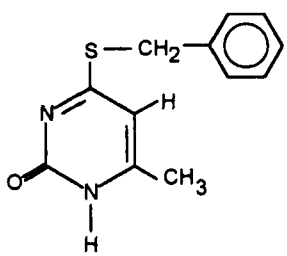
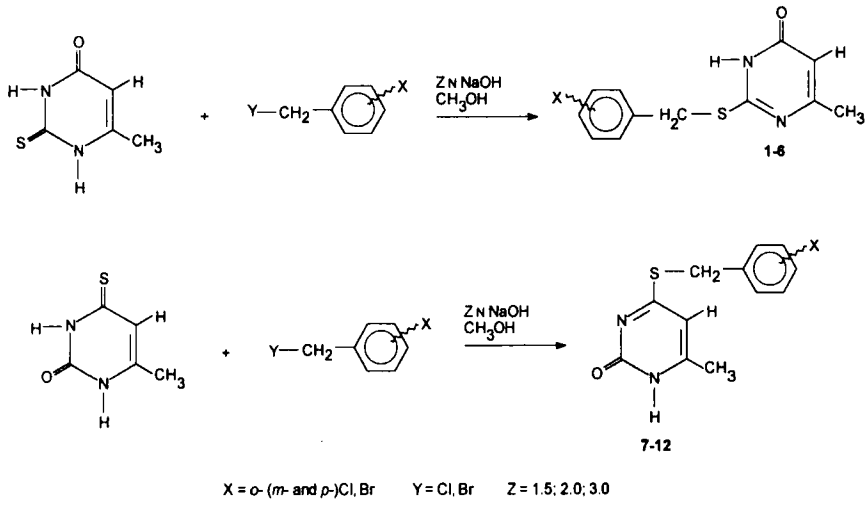


7 - 12

1 X = *o* - Cl
2 X = *m* - Cl
3 X = *p* - Cl
4 X = *o* - Br
5 X = *m* - Br
6 X = *p* - Br

7 X = *o* - Cl
8 X = *m* - Cl
9 X = *p* - Cl
10 X = *o*- Br
11 X = *m* - Br
12 X = *p* - Br

FIGURE 1 Structures of substituted uracils **1–12**.



SCHEME I

The sulfur-containing compounds were confirmed by examining their UV/VIS, IR and ^{1}H NMR spectra (Table IV), as well as by elemental analyses (Table III). The ^{1}H NMR spectra of these compounds show the characteristic resonances of the corresponding methylene groups present on sulfur. A singlet was observed at δ 4.33–4.48 ppm due to these CH_2 protons. The IR spectra of **1–12** show absorption bands of medium intensities in the region 2700–2750 cm^{-1} and which have been assigned to ν C–S vibrations.

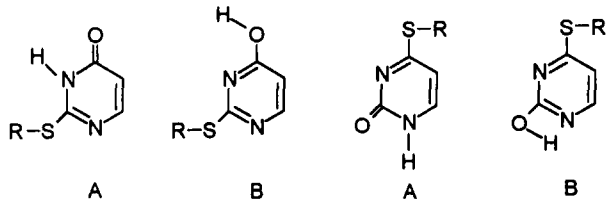
The obtained S2- and S4-benzylthio-6-methyluracils (**1–12**) may appear in various tautomeric forms differing by the positions of the protons[15]. The prototropic tautomerism of thiouracils and their S-substituted derivatives has attracted attention in the last decade[16–22]. This tautomerism is somewhat different than that of uracils. At the presently applied level of theory Leś and Adamowicz concluded[21,22] that the substitution of exocyclic oxygen by sulfur atoms in thiouracils makes the tautomerization process easier than in unsubstituted uracil. The experimental[16–22] and theoretical efforts[17–22] were directed toward determination of the tautomeric equilibria and numerous physicochemical properties of possible tautomeric forms of thiouracils and S-substituted thiouracils. It has been noted that the relative population of these forms strongly depends on the environment. In particular the form dominating in the gas phase, or in the nonpolar solvents may completely disappear in the crystaline state, or in the polar solvents and may be replaced by another tautomeric form. According to the experimental work of Rostkowska, Barski, Szczepaniak, Szcześniak and Person[16] dealing with the investigation of the IR absorption of 2-thiouracil and its

N1-, N3-, O- and S-alkyl substituted derivatives, isolated in inert low-temperature matrices, as well as the UV spectra measured in the vapor phase—2-methylthiouracil isolated in inert matrices and in the vapor adopts both the 4-oxo and 4-hydroxy tautomeric forms. The equilibrium constant K(oxo = hydroxy) is 1.2. This compound exists only as the 4-oxo form in solutions and in the solid. In the next paper Rostkowska, Szczepaniak, Nowak, Leszczyński KuBulat and Person[17] studied the infrared spectra of 2-thio and 4-thiouracils together with their N1-, N3-, O- and S-methylated derivatives isolated in low-temperature inert matrices. An assignment of the observed IR bands has been proposed on the comparison of the matrix spectra and the spectra calculated using ab initio methods. The ratio of concentrations of the tautomers K(o/h = [(oxo)/(hydroxy)] and the free energy differences ΔG, were experimentally estimated. The obtained data demonstrated that 2-methylthiouracil and 4-methylthio-6-methyluracil adopt both hydroxy and oxo tautomeric forms under the same matrix (and vapor) conditions. For 2-methylthiouracil the oxo tautomer predominates while for 4-methylthio-6-methyluracil the hydroxy form dominates.

Katritzky and coworkers[18–20] studied the tautomeric equilibria of 2-thiouracil and of four of the monoalkyl derivatives of these compounds by physical and theoretical methods (AM1 and *ab initio* calculations). They established that 2-methylthiouracil adopts two forms in tautomeric equilibrium in the gas phase. Their calculations predict that tautomer oxo is more stable than tautomer hydroxy.

Leś and Adamowicz[21,22] on the basis of the *ab initio* quantum mechanical studies of the gas-phase protomeric tautomerism of thiouracils and their methyl derivatives estimated the relative temperature-dependent distribution of various tautomeric forms in the gas phase and predicted the environmental influence on the tautomeric equilibrium. According to the conclusions from their work[21,22] 4-methylthiouracil vapor should characterize a coexistence of the hydroxy and oxo forms, with a clear predominance of the hydroxy form. A nearly equimolar hydroxy/oxo mixture should exist in the gas phase of 2-methylthiouracil. In the polar environment, however, the hydroxy—oxo tautomeric equilibrium should be strongly shifted toward the oxo form. Leś and Adamowicz[21] have predicted that corresponding to the temperature $\approx$ 500 K the main tautomeric forms in the cases of 2- and 4-methylthiouracils are forms B.

In the case of 2-methylthiouracil the tautomeric equilibrium is 1 : 10 (A : B). In the case of 4-methylthiouracil only less than 10% of the tautomeric form A is present in the tautomeric equilibrium. According to this information it is possible to suggest that 2-benzylthio-6-methyluracils (**1–6**) and 4-benzylthio-6-



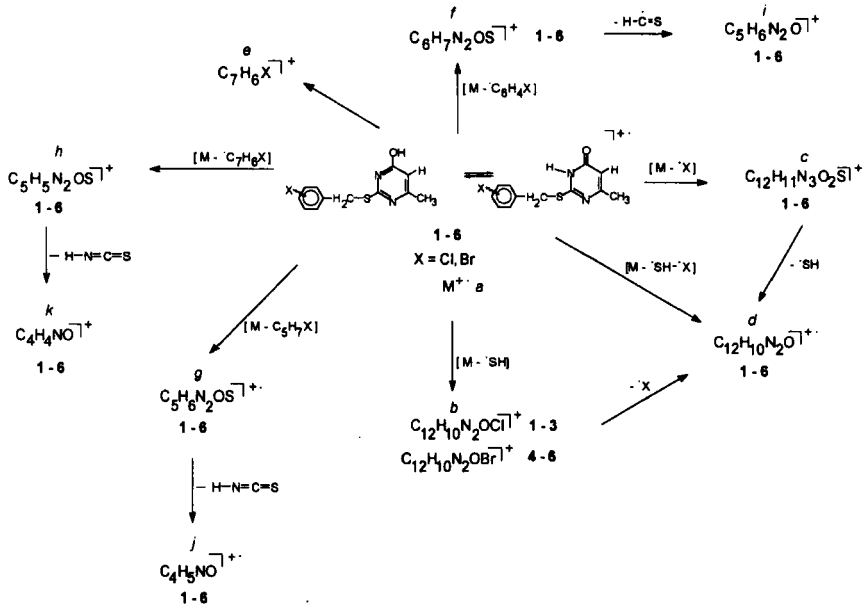
methyluracils (**7–12**) vapor should be characterized by a predominance of the hydroxy forms (B).

ELECTRON-IMPACT MASS SPECTRA

Based on metastable transitions and exact mass determinations (Table I), the principal mass spectral fragmentation routes of compounds **1–6** are interpreted as shown in Scheme 2 and those of **7–12** in Scheme 3. As can be seen from Schemes 1 and 2 the data presented in Table I for the principal mass fragmentation pathways of 2-benzylthio-6-methyluracils (**1–6**) are similar to those of the isomeric 4-benzylthio-6-methyluracils (**7–12**), but there are differences in the abundances of the important fragment ions. The common features of the mass spectral fragmentation of the molecular ions of **1–6** and **7–12** are simple cleav-

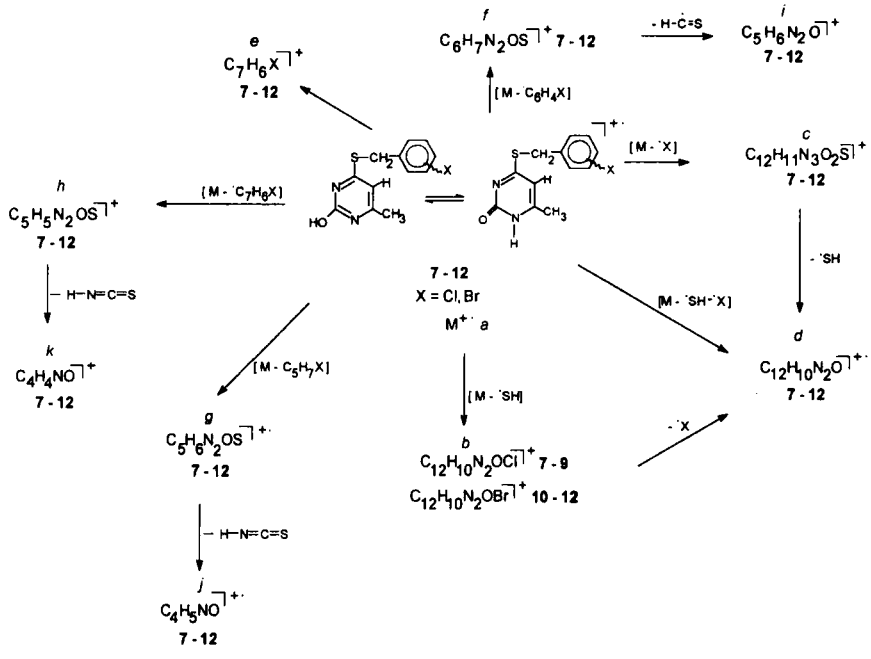
TABLE I Elemental compositions and relative intensities of the ion peaks in the spectra of 1–12 according to high resolution data

			Relative intensity (%)											
Ion	m/z	*Elemental composition*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
M^+	266	$C_{12}H_{11}N_2OSCl$	100	100	100	—	—	—	50	100	100	—	—	—
a	310/312	$C_{12}H_{11}N_2OSBr$	—	—	—	99/100	52/53	99/100	—	—	—	28/29	99/100	99/100
b	233	$C_{12}H_{10}N_2OCl$	22	54	51	—	—	—	9	23	19	—	—	—
	277/279	$C_{12}H_{10}N_2OBr$	—	—	—	3/4	5/6	31/32	—	—	—	1/1	6/7	5/5
c	231	$C_{12}H_{11}N_2OS$	66	5	3	55	65	4	100	3	3	100	7	4
d	198	$C_{12}H_{10}N_2O$	73	26	37	68	100	75	36	29	31	60	80	98
e	169/171	C_7H_6Br	—	—	—	46/47	47/48	68/69	—	—	—	26/27	32/33	65/66
	125	C_7H_6Cl	80	40	93	—	—	—	65	53	98	—	—	—
f	155	$C_6H_7N_2OS$	17	17	12	10	11	12	12	18	10	11	23	14
g	142	$C_5H_6N_2OS$	4	3	3	4	6	4	4	6	4	4	6	8
h	141	$C_5H_5N_2OS$	1	1	1	2	1	2	8	8	5	8	10	9
i	110	$C_5H_6N_2O$	23	37	31	21	21	42	8	20	16	6	26	25
j	83	C_4H_5NO	10	13	12	11	12	15	1	2	2	1	4	2
k	82	C_4H_4NO	12	18	15	10	15	18	10	23	16	9	27	29



SCHEME 2

ages of C_{sp2}-X and C_{sp3}-S bonds in the benzylthio substituent, i.e. elimination of C_7H_6X and X radicals. By these fragmentation routes the even-electron fragment ions **h** and **c** and also **e** are derived with metastable peaks for the corresponding transitions. It should to be mentioned that in the cases of **1–12** during the processes of the cleavages of C_{sp3}-S bonds of the benzylthio substituent, the positive charge is stabilized more effectively on the benzyl than on the uracil fragment. The peaks of the ions **e** are therefore in the cases **1–6** about 24–80 times more intense than those of **h** ions. In the series of 4-benzylthio-6-methyluracils (**7–12**), these differences are lower. The even-electron fragment ions **e** are only 4–20 times more intense than those of even-electron fragment ions **h**. It was also found that even-electron fragment ions **c** are the base peaks of the spectra **7** and **10**, and, in the case of **1**, the peak of this ion has the highest intensity in the series *ortho-*, *meta-* and *para*-chlorosubstituted isomers (**1–3**). Hence, it is obvious that the eliminations of ·X in the case of **1–7** and **10** are strongly connected with the ortho effect. The loss of a substituent radical from the ortho position of the phenyl ring is greatly favored because it involves the formation of a very stable even-electron tricyclic characterized by the quaternization of N (1) or N (2) (**7–10**) of the pyrimidynyl moiety (Fig. 2). The only exception is seen in the series 2- *ortho*-(*meta-* and *para*-) bromobenzylthio-6-methyluracils (**4–6**), because in this series the even-electron fragment ion **c** has



SCHEME 3

the highest intensity in the mass spectrum of *2-meta*-bromobenzylthio-6-methyluracil (**5**). It should be pointed out that the base ion in the mass spectrum of **5** is the odd-electron fragment ion **d**, which has been obtained by two simultaneous eliminations of ·SH radical from ion **c**, as well as ·SH and ·X radicals from the molecular ion **a**. The conceivable tricyclic structure of odd-electron fragment ion **d** is possible in the case of **5** the competitive stability with even-electron fragment ion **c** (Fig. 2). The molecular ions of all compounds investigated readily lose ·SH radicals, giving even-electron ions **c**. For this loss to occur, skeletal rearrangement is required with the formation of new carbon-carbon and carbon-nitrogen bonds. The even-electron fragment ions **c** which are formed after this rearrangement may have a monocyclic or bicyclic structure. It should be mentioned that loss of a sulphydryl radical is common for aromatic thioethers.[15] In the mass spectra of **1–12** are also the odd-electron fragment ions **d**, which are obtained by successive or simultaneous eliminations of X· and ·SH radicals. The appearance of even-electron ions **f** could be explained in terms of elimination of ·C_6H_4X radicals.

It should be pointed out that the base peaks of the mass spectra of **1–4**, **6**, **8**, **9**, **11**, **12** are the molecular ions **a**, and the odd-electron ion **d** is the base peak of **5**. It was also found that odd-electron fragment ions **i** are formed from the

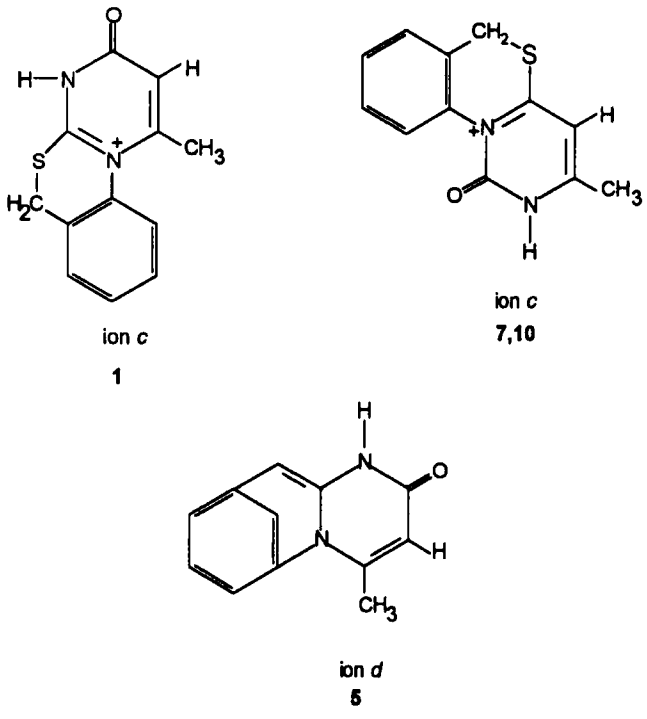


FIGURE 2 Suggested structures of even-electron fragment ions *c* and odd-electron fragment.

molecular ions by the ejection of C_7H_5X neutral molecules. The cleavage of the C-S bond probably follow a McLafferty type of rearrangement with hydrogen transfer either to sulphur or the ring nitrogen atoms.

Table II presents for all the compounds investigated (**1–12**) the ratios of the intensities of the **b**, **c**, **d**, and **e** ion peaks to those of the parent ion peaks, i.e

$$\mu_1 = \text{int. } [M\text{-}SH]^+/\text{int. } M^{+\cdot} \qquad \mu_1 = \text{int. } \mathbf{b}^+/\text{int. } \mathbf{a}^{+\cdot}$$
$$\mu_2 = \text{int. } [M\text{-}X]^+/\text{int. } M^{+\cdot} \qquad \mu_2 = \text{int. } \mathbf{c}^+/\text{int. } \mathbf{a}^{+\cdot}$$
$$\mu_3 = \text{int. } [M\text{-}SH\text{-}X]^+/\text{int. } M^{+\cdot} \qquad \mu_3 = \text{int. } \mathbf{d}^+/\text{int. } \mathbf{a}^{+\cdot}$$
$$\mu_4 = \text{int. } C_7H_6X^+/\text{int. } M^{+\cdot} \qquad \mu_4 = \text{int. } \mathbf{e}^+/\text{int. } \mathbf{a}^{+\cdot}$$

As can be seen from the data in Table II, the differences between the relative intensities of the peak of the selected fragment ions **b–c** and $M^{+\cdot}$ **a** ions, i.e the values of μ_1–μ_4 for 2- (and 4-) *o*- (and *p*-) chloro- (and bromo-)benzylthiouracils may be sufficient to differentiate isomers. It is possible to distinguish isomeric 2- and 4-benzylthiouracils, and also isomeric *ortho*-, *meta*- and *para*-substituted benzylthiouracils. This problem seems not to have been tackled previously. The

TABLE II Values of μ_1–μ_4 calculated from the EI mass spectra recorded at 75 eV of 2-chlorobenzylthio-6-methyluracils (**1–3**), 2-bromobenzylthio-6-methyluracils (**4–6**), 4-chlorobenzylthio-6-methyluracils (**7–9**), 4-bromobenzylthio-6-methyluracils (**10–12**)

Compound	*Substitution*	μ_1	μ_2	μ_3	μ_4
1	ortho	0.22	0.66	0.77	0.80
2	meta	0.54	0.05	0.26	0.40
3	para	0.51	0.03	0.37	0.93
4	ortho	0.04	0.55	0.68	0.47
5	meta	0.11	1.22	1.88	0.90
6	para	0.32	0.04	0.75	0.69
7	ortho	0.18	2.00	0.72	1.30
8	meta	0.23	0.03	0.29	0.53
9	para	0.19	0.03	0.31	0.98
10	ortho	0.03	3.44	2.06	0.93
11	meta	0.07	0.07	0.80	0.33
12	para	0.05	0.04	0.98	0.66

2-substituted isomers of *o*-(*m*- and *p*-) chlorobenzylthio-6-methyluracils (**1–3**) may be distinguished from isomeric 4-substituted compounds (**7–9**) on the basis of the higher values of μ_1. The highest values of μ_2 and μ_3 are useful for the differentiation of *o*-chloro-substituted isomers (**1**, **7**) in the serie of 2- and 4-*o*-(*m*- and *p*-)chlorobenzylthio-6-methyluracils (**1–3**, **7–9**) according to the following sequences-

$$\mu_2\ (ortho) > \mu_2\ (meta) \text{ and } (para)$$

$$\mu_3\ (ortho) > \mu_3\ (meta) \text{ and } (para)$$

In the same series (**1–3**, **7–9**) *p*-chloro-substituted isomers (**3**, **9**) may be distinguished from the *meta* isomers on the basis of the higher values of μ_4. The values of μ_2–μ_4 are useful for differentiation of 2- and 4-substituted *o*- as well as *m*-bromobenzylthio-6-methyluracils (**4**, **5**, **10**, **11**). In the case of **4** the values of μ_2–μ_4 are lower, and in the case of **5** higher, than those which have been observed in the case of corresponding **10** and **11**. The 2-*p*-bromobenzylthio-substituted isomer **6** has however, the higher value of μ_1 than corresponding 4-substituted counterpart **12**. In the series *o*-(*m*- and *p*-)bromo substituted benzylthio-6-methyluracils (**4–6**, **10–12**) it is also possible to use the values of μ_3 and μ_4 in the differentiation of the position of the substitution of the Br atom in the phenyl ring of benzylthio substituent according to the following sequences:

$$\textbf{4–6}\ \mu_3, \mu_4\ (meta) > \mu_3, \mu_4\ (para) > \mu_3, \mu_4\ (ortho)$$

TABLE III Physical and analytical data of compounds **1–12**

Comp.	*Formula Mol. mass*	*Yield %*	*m.p.°C*	*Solvent for cryst.*	*TLC R_f $CHCl_3$:MeOH (5:1)*	*Analysis % Calcd./Found*		
1	$C_{12}H_{11}N_2OSCl$	84.4	210–1	a	0.54	54.06	4.16	10.51
	266.03					54.09	3.93	10.30
2	$C_{12}H_{11}N_2OSCl$	63.2	172–3	a	0.52	54.06	4.16	10.51
	266.03					53.73	3.96	10.36
3	$C_{12}H_{11}N_2OSCl$	72.4	185–6	a	0.55	54.06	4.16	10.51
	266.03					53.84	3.94	10.29
4	$C_{12}H_{11}N_2OSBr$	68.6	216–8	a	0.53	46.31	3.56	9.00
	311					46.26	3.56	8.98
5	$C_{12}H_{11}N_2OSBr$	57.1	222–4	a	0.51	46.31	3.56	9.00
	311					46.23	3.51	8.95
6	$C_{12}H_{11}N_2OSBr$	63.3	189–91	a	0.55	46.31	3.56	9.00
	311					46.26	3.42	8.73
7	$C_{12}H_{11}N_2OSCl$	58.8	179–82	b	0.48	54.06	4.16	10.51
	266.03					53.85	4.04	10.50
8	$C_{12}H_{11}N_2OSCl$	47.6	149–51	b	0.44	54.06	4.16	10.51
	266.03					53.88	3.86	10.22
9	$C_{12}H_{11}N_2OSCl$	51.7	158–61	b	0.50	54.06	4.16	10.51
	266.03					53.93	4.05	10.13
10	$C_{12}H_{11}N_2OSBr$	49.8	147–9	b	0.56	46.31	3.56	9.00
	311					45.99	3.47	8.87
11	$C_{12}H_{11}N_2OSBr$	41.0	175–7	b	0.51	46.31	3.56	9.00
	311					45.98	3.21	9.00
12	$C_{12}H_{11}N_2OSBr$	43.8	159–61	b	0.57	46.31	3.56	9.00
	311					46.20	3.38	8.89

a—methanol
b—ethanol

10–12 μ_3, μ_4 (*ortho*) > μ_3, μ_4 (*para*) > μ_3, μ_4 (*meta*)

EXPERIMENTAL

The purity of all described compounds was monitored by m.p.'s and T.L.C. The melting points were determined on a Böetius apparatus. R_f values refer to TLC plates with silica gel F_{254} (Merck) developed with $CHCl_3/CH_3OH$ (5:1) and observed under UV light (λ = 254 and 366 nm). IR spectra were recorded on a Perkin-Elmer M-180 Spectrophotometer in KBr pellets. UV/VIS spectra were recorded on a Specord UV/VIS Spectrophotometer in methanol. ^1H NMR spectra were recorded on a Varian Gemini 300 spectrometer at 75 MHz in DMSO-$_{d6}$

TABLE IV ^{1}H NMR, IR and UV/VIS spectral data of compounds **1–12**

Compound	*^{1}H NMR (δ, ppm)*			*IR (cm^{-1})*	*UV/Vis*	
	S-CH_2(s)	*C_5-H(s)*	*C_6CH_3(s)*	*ν C=O (ν S-CH_2)*	*λ_{max}(nm)*	*lgε*
1	4.48	6.02	2.23	1680	277.0	3.86
				(2740)	230.2	3.97
2	4.38	6.01	2.21	1660	277.2	3.87
				(2730)	229.2	4.01
3	4.37	6.00	2.20	1655	270.0	3.89
				(2740)	231.4	4.02
4	4.48	6.02	2.24	1685	276.4	3.87
				(2740)	223.8	4.19
5	4.48	6.02	2.23	1680	277.0	3.86
				(2740)	231.2	3.98
6	4.36	6.01	2.21	1655	276.2	3.52
				(2735)	227.4	3.91
7	4.46	6.21	2.12	1695	297.6	4.03
				(2795)	269.2	3.96
					221.0	3.94
8	4.38	6.21	2.12	1670	297.8	3.99
				(2790)	268.6	3.93
					221.2	3.90
9	4.37	6.21	2.12	1670	298.0	3.99
				(2790)	268.4	3.92
					225.0	3.94
10	4.45	6.21	2.12	1670	299.4	4.08
				(2790)	271.6	3.97
					216.2	413
11	4.38	6.22	2.13	1665	299.8	4.07
				(2750)	271.8	3.96
					216.2	4.16
12	4.35	6.20	2.12	1670	299.4	4.05
				(2760)	269.6	3.95
					220.0	4.13

solution with TMS as the internal standard. All chemical shifts are quoted in δ values. Low and high resolution mass spectra were recorded on a Jeol JMS-D-100 mass spectrometer linked to a Texas Instruments 980 B computer (ionizing voltage 75 eV, ionizing current 300 μA, accelerating voltage 3KV, resolution 10000). Low resolution mass spectra were also recorded on LKB 2091 mass spectrometer (ionizing voltage 15 and 75 eV, ionizing current 300 μA, accelerating voltage 3,5 KV). The elemental compositions of the ions was also determined by peak matching relative to perflorokerosene. All measured masses agreed with those of the compositions listed in column 3 of Table I to within ±

2 ppm. Metastable transitions were measured in the first field-free region on the same instrument with a metastable ion detector using a high-voltage scan. The values of μ_1–μ_4 were calculated as averages of two to four measurements.

Synthesis of 2-ortho-(meta- and para-)chlorobenzylthio-6-methyluracils (*1–3*).

A methanol solution consisting of 5 mmoles of 2-thio-6-methyluracil in 50 ml of 0.3 N NaOH was stirred at room temperature while 10 mmoles of corresponding chlorobenzyl chloride was added dropwise. After stirring for 20 hrs the reaction mixture was acidified (pH 3) with 6 M HCl. The precipitated solid was filtered, washed with water and dried at room temperature under vacuum. Recrystallization from methanol afforded the analytical samples of compounds **1–3** (Table I).

Since the same method was applied for the synthesis of all new 2- and 4-benzylthio-6-methyluracils (**1–12**), the conditions of the remaining preparations of these compounds have been summarized in Table V.

TABLE V The conditions of the preparations of compounds **4–12**

Compound	*Substrates-mmoles*	*NaOH (N)*
4	a — 5 c — 7.5	0.15
5	a — 5 c — 7.5	0.3
6	a — 5 c — 7.5	0.3
7	a — 5 b — 10	0.2
8	a — 5 b — 10	0.2
9	a — 5 b — 10	0.2
10	a — 5 c — 7.5	0.15
11	a — 5 c — 7.5	0.3
12	a — 5 c — 7.5	0.3

a—2-(or 4-)thio-6-methyluracil
b—corresponding *o*-(*m*- and *p*-)chlorobenzyl chloride
c—corresponding *o*- (*m*- and *p*-)bromobenzyl bromide

References

[1] W. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag; New York, Berlin, Heidelberg, Tokyo, 1984, chapter 7.
[2] *The Pharmacological Basis of Therapeutics*, L. S. Goodman, A. Gilman 5-th Ed. Macmillan, New York, 1975.
[3] U. Thewald and C. E. Bugg, *J. Am. Chem. Soc.*, **94**, 8892 (1972).
[4] W. Saenger and D. Suck, *Eur. J. Biochem.*, **82**, 473 (1973).
[5] A. G. Lezius and K. H. Scheit, *Eur. J. Biochem.*, **3**, 85 (1961).
[6] K. H. Scheit and E. Gartner, *Biochim. Biophys. Acta*, **182**, 10 (1969).
[7] E. Gottschalk, E. Kopp and A. G. Lezius, *Eur. J. Biochem.*, **24**, 168 (1971).
[8] P. Crain, T. Hasizume and J. A. McCloskey, *In Biological Mass Spectrometry*, ed. by A. L. Burlingame and J. A. McCloskey, p. 671–692 (1990), Elsevier, Amsterdam.
[9] K. Schram, *In Biomedical Applications of Mass Spectrometry* ed. by C. H. Suelter and J. T. Watson, p. 203–288, Wiley, New York (1990).
[10] J. A. McCloskey, *In Mass Spectrometry in the Health and Life Sciences*, ed. by A. L. Burlinguame and N. Castagnoli Jr., Elsevier, Amsterdam (1985).
[11] J. A. McCloskey, In *Mass Spectrometry in Biomedical Research*, ed. by S. J. Gaskell, Wiley, New York (1986).
[12] E. Wyrzykiewicz, G. Barkowiak, Z. Nowakowska and B. Kędzia, *Il Farmaco*, **48(7)**, 979 (1993).
[13] E. Wyrzykiewicz and G. Bartkowiak, *Organic Mass Spectrometry*, **27**, 1377 (1992).
[14] E. Wyrzykiewicz and G. Bartkowiak, *Organic Mass Spectrometry*, **29**, 50 (1994).
[15] J. Elguero, C. Marzin, A. R. Katritzky and P. Linda, *The Tautomerism of Heterocycles* eds. A. R. Katritzky and A. J. Boulton, Academic Press, London (1976).
[16] H. Rostkowska, A. Barski, K. Szczepaniak, M. Szcześniak and W. B. Person, *Journal of Molecular Structure*, **176**, 137 (1988).
[17] H. Rostkowska, K. Szczepaniak, M. J. Nowak, J. Leszczyński, K. KuBulat and W. B. Person, *J. Am. Chem. Soc.*, **112**, 2147 (1994) and literature cited therein.
[18] A. R. Katritzky, G. Baykut, S. Rachwal, M. Szafran, K. C. Caster and J. Eyler, *J. Chem. Soc. Perkin Trans. II*, 1499 (1989).
[19] A. R. Katritzky, M. Szafran and J. Stevens, *J. Chem. Soc. Perkin Trans. II*, 1507 (1989).
[20] A. R. Katritzky, M. Szafran and G. Pfister-Guillonzo, *J. Chem. Soc. Perkin Trans. II*, 871 (1990).
[21] A. Leś and L. Adamowicz, *J. Am. Chem. Soc.*, **112**, 1504 (1990).
[22] A. Leś L. Adamowicz, *J. Phys. Chem.*, **94**, 7021 (1990) and literature cited therein.
[23] H. H. Beynon, R. A. Saunders and A. E. Williams, *The Mass Spectra of Organic Molecules*, p. 363 (1968), Elseviel, Amsterdam.