

Elżbieta Wyrzykiewicz *, Monika Wendzonka

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

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Twelve new fluorescent (*E*)-2-stilbenyloxyalkylthiouracils and 6-methyluracils **5a-5l** were prepared. EI induced mass spectral fragmentation of these compounds was investigated. Fragmentation pathways are proposed on the basis of accurate mass and metastable transition measurements. Correlation between the intensities of the M^{+} and the selected fragment ions of these compounds is discussed. The data obtained permit a distinction of the metamers. The ^1H and ^{13}C NMR spectra of these compounds were assigned unambiguously using a combination of heteronuclear (HETCOR) spectra and the chemical shifts. The data derived from these spectra can be used to differentiate the isomers.

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Introduction.

Thio derivatives of pyrimidine bases are of interest because of their biological and pharmacological activities [1-7]. Many of the modified nucleobases and nucleosides both with additional five- and six-membered rings are fluorescent [8-11] and offer a possibility of gaining information concerning DNA and RNA structure and dynamic [12-14]. Many have been shown to enter biochemical pathways and have given indications about their binding to different enzymes [15-16]. The fluorescent derivatives of cytosine and cytidine have been widely reported in the literature [11,17-20]. However, to the best of our knowledge, very little work has been published on the synthesis and physicochemical properties of fluorescent derivatives of 2-thiouracils. This fact has stimulated us to prepare a series of (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5e,5g,5i,5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**) with the idea that these compounds bearing on pyrimidine ring (*E*)-stilbenyloxyalkylthio substituents with strongly fluorescent (*E*)-stilbene moiety, would certainly be fluorescent [21,22].

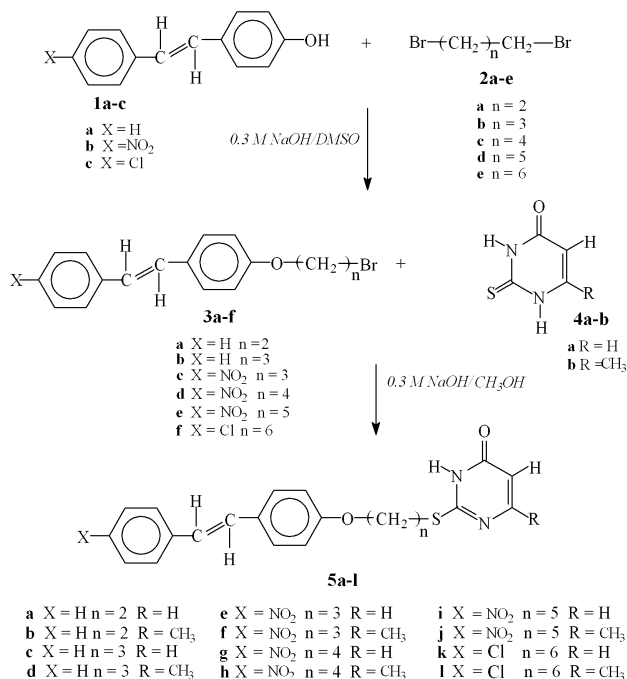
This paper deals with the synthesis and physicochemical properties of **5a-5l**. The UV/VIS, IR, ^1H NMR and ^{13}C NMR of (*E*)-bromoalkoxystilbenes (**3a-3f**) have also been investigated because these compounds are also unknown in the literature. The analyses of ^1H NMR, ^{13}C NMR and EI mass spectra of **5a-5l** have been performed to check the possibility of differentiation of (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5e,5g,5i,5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**). The investigation of electron-impact induced mass spectra was also undertaken to discover whether it is possible to distinguish the metamers with molecular formulas $\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$ (**5b,5c**); $\text{C}_{22}\text{H}_{21}\text{N}_3\text{O}_4\text{S}$ (**5f,5g**) and $\text{C}_{23}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$ (**5h,5i**) on the basis of differences in values of μ_1 and μ_2 i.e. the ratio of the intensity of selected fragment ion peaks to that of the parent ion peak, and to compare the data with those previously obtained in our laboratory [23-25].

Results and Discussion.

A few series of twelve new fluorescent (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5e,5g,5i,5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**) have been synthesized in the reaction of 2-thiouracil (or 2-thio-6-methyluracil) with corresponding (*E*)-bromoalkoxystilbenes **3a-3f**. Treatment of 2-thiouracil (or 2-thio-6-methyluracil) with **3a-3f** in 0.3 *M* solution of NaOH in methanol at room temperatures afforded **5a-5l** (Scheme 1). A series of new **3a-3f** have been synthesized in the reaction of (*E*)-stilbenol-4, [(*E*)-4'-chlorostilbenol-4, (*E*) 4'-nitrostilbenol-4)] with 1,2-dibromoethane (1,3-dibromopropane; 1,4-dibromobutane; 1,5-dibromopentane; 1,6-dibromohexane) in 0.3 *M* NaOH solution of DMSO at room temperature. The structures of all compounds obtained were determined by examining their UV/VIS, IR, ^1H NMR and ^{13}C NMR spectra as well as on the basis of elemental analyses (Tables 1-7).

The ^1H and ^{13}C NMR data of **3a-3f** and **5a-5l** are given in Tables 3-7. Assignments of the ^1H NMR and ^{13}C NMR resonances of these compounds were deduced on the basis of signal multiplicities, and by the concerted application of the two-dimensional NMR technique HETCOR. The HETCOR results allow unequivocal assignment of the ^{13}C NMR spectra proposed on the basis of chemical shifts theory, additivity rules and by comparing the measured and calculated chemical shifts [31]. (*E*) configuration in the stilbene part of the molecules of **3a-3f** and **5a-5l** was determined on the basis of their UV/VIS and IR spectra. It has been pointed out that in the UV/VIS spectra of **3a-3f** λ_{max} are in the range 320-378 nm (Table 3), as well as **5a-5l** 318.5-362.5 nm (Table 4) respectively. According to the literature [26-28] (*E*)-stilbenes exhibited the values of λ_{max} in range 290-360 nm and for (*Z*)-stilbenes values of λ_{max} fall in the range 260-280 nm. The infrared spectra of **3a-3f** and **5a-5l** show a strong band in the range 960-987 cm^{-1} which according to the literature [29-30] can be attributed to the out-of-plane deformation vibration of the C-H bond of the (*E*)-ethylene bridge of the stilbene skeleton (Tables 3,4).

Scheme 1



In the ¹H NMR spectra of (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5e,5g,5i,5k**) are seen two doublets of CV-H and CVI-H protons of uracil ring. The values of the chemical shifts of the signals of these protons were established in the range 6.52-6.60 δ and 7.45-8.00 δ, respectively (Table 4). In the ¹H NMR spectra of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**) are seen two singlets of CV-H and CVI-CH₃ protons of 6-methyluracil ring. The values of the chemical shifts of the signals of these protons fall in the range 6.37-6.83 δ and 2.24-2.49 δ, respectively. The presence of these signals in the ¹H NMR spectra of 2-(*E*)-stilbenyloxyalkylthiouracils and 2-(*E*)-stilbenyloxyalkylthio-6-methyluracils allows for the determination of the presence of uracil or 6-methyluracil

ring in the molecules of **5a-5l**. The determination of the presence of 6-methyl-uracil ring in the molecules of **5b, 5d, 5f, 5h, 5j** and **5l** is also possible on the basis of the presence in the ¹³C NMR spectra of these compounds the signals of carbons of C VI -methyl group of uracil ring. The values of the chemical shifts of the signals of these carbons fall in the range 18.94-24.23 δ.

A comparison of the number and positions of the C II, C IV, C VI carbon atom signals in the region 160-170 ppm of ¹³C NMR spectra of (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5e,5g,5i,5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**) allows a differentiation between metameric **5b** and **5c** [C₂₁H₂₀N₂O₂S], **5f** and **5g** [C₂₂H₂₁N₃O₄S] as well as **5h** and **5i** [C₂₃H₂₃N₃O₄S]. This data are given in tabular form below:

5b (*E*)-2-[stilbenyl-4-oxyethylthio-6-methyluracil];

C II 167.05 ppm

C IV 160.63 ppm

C VI 162.24 ppm

5c (*E*)-2-[stilbenyl-4-oxypropylthiouracil]

C II 166.40 ppm

C IV 161.56 ppm

C VI 149.76 ppm

5f (*E*)-2-[4'-nitrostilbenyl-4-oxypropylthio-], 6-methyluracil,

C II 165.70 ppm

C IV 164.56 ppm

C VI 160.01 ppm

5g (*E*)-2-[4'-nitrostilbenyl-4-oxybutylthio-]uracil

C II 161.70 ppm

C IV 160.89 ppm

C VI 146.18 ppm

5h (*E*)-2-[4'-nitrostilbenyl-4-oxybutylthio-], 6-methyluracil

C II 165.70 ppm

C IV 164.56 ppm

C VI 160.30 ppm

Table 1

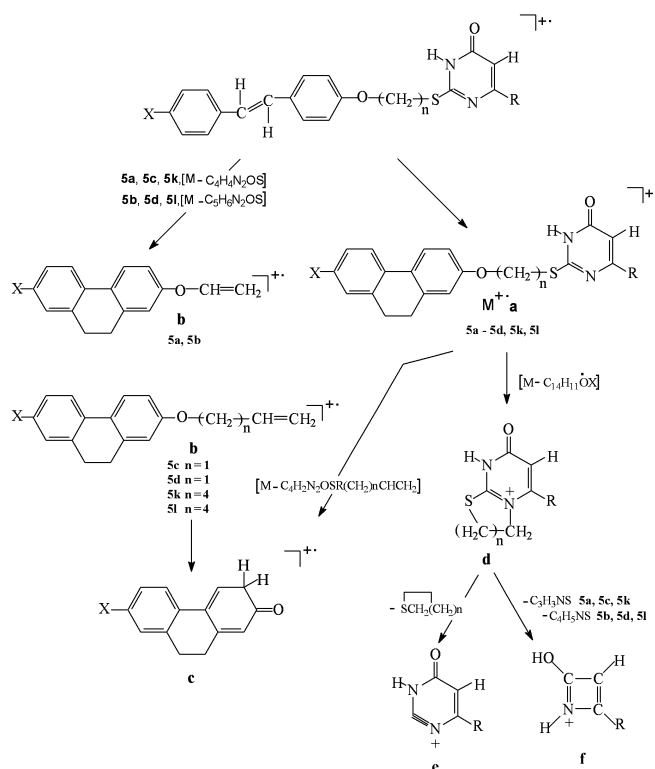
Chemical and Physical Data of Compounds **3a-f**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield %	R _f TLC	Elemental analysis		
					Calculated (Found)	C	H
3a	C ₁₆ H ₁₅ OBr 303.20	123-4	72	0.50	63.36 (63.13)	4.95 (5.15)	-
3b	C ₁₇ H ₁₇ OBr 317.22	108-9	62	0.52	64.35 (63.74)	4.95 (5.23)	-
3c	C ₁₇ H ₁₆ NO ₃ Br 362.23	72-4	30	0.74	56.35 (56.15)	4.42 (4.62)	3.84 (3.60)
3d	C ₁₈ H ₁₈ NO ₃ Br 376.25	82-3	16	0.74	57.45 (57.85)	4.79 (4.80)	3.72 (3.78)
3e	C ₁₉ H ₂₀ NO ₃ Br 408.25	77-8	17	0.72	55.88 (55.65)	5.39 (5.11)	3.43 (3.41)
3f	C ₂₀ H ₂₂ ONBrCl 394.20	136-8	72	0.51	60.91 (60.60)	6.69 (6.73)	-

Table 2
Chemical and Physical Data of Compounds **5a-l**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield %	R _f TLC	Elemental analysis			
					Calculated (Found)	C	H	N
5a	C ₂₀ H ₁₈ N ₂ O ₂ S 350.06 x 1/2 H ₂ O	246-8	20	0.73	66.85 (66.77)	5.01 (4.94)	7.79 (7.60)	8.91 (8.82)
5b	C ₂₁ H ₂₀ N ₂ O ₂ S 364.04	213-5	53	0.59	69.23 (66.92)	5.49 (5.56)	7.69 (7.40)	8.79 (8.70)
5c	C ₂₁ H ₂₀ N ₂ O ₂ S 364.04	222-3	19	0.66	69.23 (69.94)	5.49 (5.59)	7.69 (7.41)	8.79 (8.62)
5d	C ₂₂ H ₂₂ N ₂ O ₂ S 405.3 x 1/2 H ₂ O	187-9	24	0.57	65.18 (65.44)	5.43 (5.52)	6.91 (6.41)	7.90 (7.82)
5e	C ₂₁ H ₁₉ N ₃ O ₄ S 409.41	93-4	73	0.48	61.61 (61.72)	4.65 (4.62)	10.26 (10.05)	7.82 (7.51)
5f	C ₂₂ H ₂₁ N ₃ O ₄ S 423.44	175-7	40	0.51	62.41 (62.58)	4.96 (5.02)	9.93 (9.98)	7.56 (7.51)
5g	C ₂₂ H ₂₁ N ₃ O ₄ S 432.44 x 1/2 H ₂ O	154-6	77	0.54	61.11 (61.04)	5.09 (5.23)	9.72 (9.41)	7.56 (7.62)
5h	C ₂₃ H ₂₃ N ₃ O ₄ S 437.46	132-4	65	0.47	63.16 (63.12)	5.26 (5.36)	9.60 (9.47)	7.32 (7.39)
5i	C ₂₃ H ₂₃ N ₃ O ₄ S 437.46	148-50	57	0.54	63.10 (63.05)	5.26 (5.24)	9.60 (9.57)	7.32 (7.40)
5j	C ₂₄ H ₂₅ N ₃ O ₄ S 451.49	139-41	81	0.57	63.86 (63.56)	5.54 (5.48)	9.31 (9.18)	7.09 (7.00)
5k	C ₂₄ H ₂₅ N ₂ O ₂ SCl 449.45 x 1/2 H ₂ O	189-91	16	0.67	62.88 (63.00)	5.89 (5.56)	6.11 (6.23)	7.12 (8.06)
5l	C ₂₅ H ₂₇ N ₂ O ₂ SCl 463.45 x 1/2 H ₂ O	169-71	19	0.65	64.79 (64.83)	5.83 (5.86)	6.04 (5.86)	6.91 (6.78)

Scheme 2



5i (E)-2-[4'-nitrostilbenyl-4-oxypentylthiouracil

C II 165.65 ppm

C IV 164.86 ppm

C VI 146.00 ppm

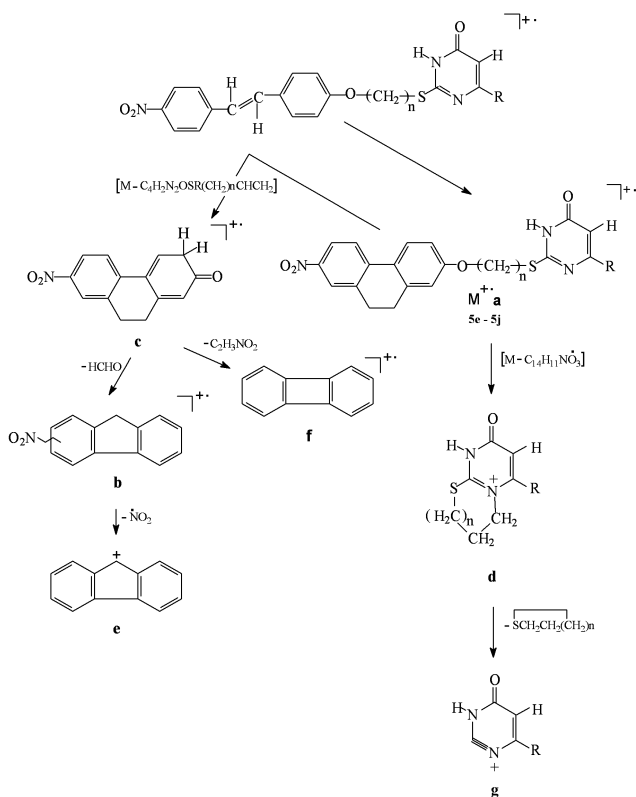
On the basis of the low and high resolution electron-impact mass spectra, as well as fragment ion spectra from the first field-free region, recorded using linked scans at constant B/E (Tables 8, 9), the principal mass spectral fragmentation routes of the molecular ions of compounds **5a-5l** are interpreted as shown in Scheme 2 and 3. The stilbenyloxyalkyl substituents of the molecular ions of **5a-5l** might have a tricyclic dihydrophenanthrenyloxyalkyl structure, as it was postulated by us earlier in the cases of the mass decompositions of the molecular ions of *N*-(*E*)-stilbenyloxyalkylcarbonyl substituted hydrazones of arylaldehydes [25,32,33]. The common features of the mass spectral fragmentation of the molecular ions of **5a-5l** are simple cleavages of Csp³-O bonds in the thioalkyloxy chain *i.e.* the elimination of stilbenyloxy radicals. By these simple inductive cleavage the even-electron fragment ions **d** are derived. These ions might have a bicyclic structure with the positive charge probably situated on the annular nitrogen atom, as was proposed earlier for the mass fragmentation of alkoxyalkylthiouracils [34].

It ought to be pointed out that cleavages of Csp²-S and Csp³-N bonds in these even-electron fragment ions leading to ejection of neutral alkenethiols molecules to give even-electron fragment ions situated in the mass spectra of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5a,5c,5e,5g,5i,5k**) at

Table 3
UV/VIS, IR and ^1H NMR Data of **3a-f**

Comp.	UV/VIS λ max	(lg ϵ)	IR (cm $^{-1}$) ν C=C	HC=CH trans	ν C-O-	^1H NMR δ (ppm)
3a	240	(3.95)	1606			OCH $_2$ 4.32 t J= 6Hz
	306	(4.43)	1595	961	1255	BrCH $_2$ 3.66 t J= 6Hz
	320	(4.42)	1511			Ar 6.88-7.51 m
3b	240	(3.97)	1603			OCH $_2$ 4.13 t J= 6Hz
	308	(4.42)	1588	968	1246	BrCH $_2$ 3.61 t J= 6Hz
	320	(4.41)	1511			Ar 6.88-7.51 m
3c	259	(4.14)	1604			OCH $_2$ 4.33 t J= 6Hz
	276	(4.12)	1588	969	1253	BrCH $_2$ 3.66 t J= 6Hz
	376	(4.30)	1511			Ar 6.88-7.51 m
3d	258	(4.08)	1604			OCH $_2$ 4.13 t J= 6Hz
	275	(4.05)	1589	974	1254	BrCH $_2$ 3.61 t J= 6Hz
	376	(4.32)	1512			Ar 6.88-7.51 m
3e	258	(4.12)	1604			OCH $_2$ 3.98 t J= 6Hz
	276	(4.09)	1589	960	1249	BrCH $_2$ 3.48 t J= 6Hz
	378	(4.35)	1510			Ar 6.86-7.52 m
3f	240	(4.05)	1605			OCH $_2$ 3.98 t J= 6Hz
	310	(4.44)	1573	968	1253	BrCH $_2$ 3.48 t J= 6Hz
	328	(4.47)	1511			Ar 6.86-7.45 m

Scheme 3



m/z 95 [$\text{C}_4\text{H}_3\text{N}_2\text{O}$] (ion **e**, Table 8, Scheme 2; ion **g** Table 9, Scheme 3) and in the mass spectra of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**, **5d**, **5f**, **5h**, **5j**, **5l**) at m/z 109 [$\text{C}_5\text{H}_5\text{N}_2\text{O}$] (ion **e**, Table 8, Scheme 2; ion **g** Table 9, Scheme 3). The presence in the mass spectra of **5b**, **5d**, **5f**,

5h, **5j** and **5l** the even-electron fragment ions situated at m/z 109 [$\text{C}_5\text{H}_5\text{N}_2\text{O}$] allows one to identify the presence of the 6-methyl group on the uracil moiety of these compounds.

It was found that the cleavage of the $\text{Csp}^3\text{-O}$ bond in the thioalkoxy chain of **5a-5l** follows also McLafferty rearrangement involving the migration of a hydrogen atom to a double bond of the phenyl ring of the (*E*)-stilbene skeleton. Odd-electron fragment ions **c** (Scheme 1) or **b** (Scheme 2) were obtained by this fragmentation pathways. The differences in the fragmentation of metameric pairs of (*E*)-2-stilbenyloxyalkylthiouracils (**5a**, **5c**, **5e**, **5g**, **5i**, **5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b**, **5d**, **5f**, **5h**, **5j**, **5l**) *i.e.* **5b** and **5c**; **5f** and **5g**; **5h** and **5i** have been quantified by comparing the calculated values of the coefficients μ_1 and μ_2 *i.e.* the abundances of the selected fragment ions relative to the abundances of the molecular ions.

$$\mu_1 = \frac{\% \text{ rel. int. } \mathbf{d}^+}{\% \text{ rel. int. } \mathbf{a}^+} \quad \mu_2 = \frac{\% \text{ rel. int. } \mathbf{c}^+}{\% \text{ rel. int. } \mathbf{a}^+}$$

It has been established by us previously that the differences in the values of coefficients μ are useful for differentiation of the positional isomers of benzylthiouracils [35-37].

As can be seen from the data in Table 10, the differences between the values of the coefficients μ_1 and μ_2 [*i.e.* the calculated quotients of the relative intensities of the selected fragment ions **d** (μ_1) and **c** (μ_2) as well as molecular ions $\text{M}^+\mathbf{a}$] are sufficient to differentiate isomers. The (*E*)-2-stilbenyloxyalkylthiouracils (**5b**, **5f**, **5h**) are distinguished from metameric (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5c**, **5g**, **5i**) on the basis of the lower values of μ_1 and the higher values of μ_2 .

Table 4
UV/VIS, IR and ¹H NMR Data of **5a-l**

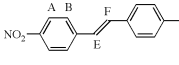
Comp.	UV/VIS λ max (lg ε)	νC=C	IR (cm ⁻¹)		νSCH ₂	¹ H NMR δ (ppm)	
			νC=O	HC=CH		 5e-j	
			trans				
5a	292.5 (4.43) 318.5 (4.47)	1605 1535 1511	1658	961	2443	OCH ₂	4.45 t J= 6Hz
						SCH ₂	3.74 t J= 6Hz
						CVI H	8.00 d J= 7Hz
						CV H	6.52 d J= 7Hz
						Ar	6.90-7.49 m
5b	292.5 (4.45) 318.5 (4.47)	1603 1577 1510	1648	962	2453	OCH ₂	4.45 t J= 6Hz
						SCH ₂	3.75 t J= 6Hz
						CV H	6.43 s
						CVI CH ₃	2.49 s
						Ar	6.93-7.51 m
5c	292.5 (4.45) 318.5 (4.44)	1602 1595 1511	1703	968	2449	OCH ₂	4.50 t J= 6Hz
						SCH ₂	3.50 t J= 6Hz
						CVI H	8.07 d J= 7Hz
						CV H	6.54 d J= 7Hz
						Ar	6.93-7.51 m
5d	292.5 (4.44) 318.5 (4.45)	1604 1592 1511	1644	967	2445	OCH ₂	4.42 t J= 6Hz
						SCH ₂	3.61 t J= 6Hz
						CV H	6.86 s
						CVI CH ₃	2.39 s
						Ar	6.93-7.51 m
5e	265.0 (4.32) 359.5 (4.17)	1603 1587 1511	1705	970	2445	OCH ₂	4.16 t J= 6Hz
						SCH ₂	3.53 t J= 6Hz
						CVI H	7.49 d J= 7Hz
						CV H	6.53 d J= 7Hz
						d _A	8.20 J=9Hz
						d _B	7.61 J=9Hz
						d _C	6.95 J=9 Hz
						d _D	9.92 J=9Hz
						d _E	6.97 J=16Hz
						d _F	7.24 J=16Hz
5f	266.0 (3.88) 359.5 (3.72)	1605 1587 1511	1635	968	2446	OCH ₂	4.19 t J= 6Hz
						SCH ₂	3.43 t J= 6Hz
						CV H	6.84 s
						CVI CH ₃	2.29 s
						d _A	8.20 J=9Hz
						d _B	7.60 J=9Hz
						d _C	6.94 J=9 Hz
						d _D	9.91 J=9Hz
						d _E	6.99 J=16Hz
						d _F	7.23 J=16Hz
5g	271.0 (4.27) 361.0 (4.08)	1605 1589 1511	1657	964	2449	OCH ₂	4.11 t J= 6Hz
						SCH ₂	3.43 t J= 6Hz
						CVI H	7.49 d J= 7Hz
						CV H	6.60 d J= 7Hz
						d _A	8.21 J=9Hz
						d _B	7.61 J=9Hz
						d _C	6.95 J=9Hz
						d _D	6.91 J=9Hz
						d _E	6.99 J=16Hz
						d _F	7.24 J=16Hz
5h	270.0 (4.29) 359.5 (4.09)	1605 1589 1512	1644	961	2453	OCH ₂	4.06 t J= 6Hz
						SCH ₂	3.45 t J= 6Hz
						CVI H	6.49 s
						CV CH ₃	2.30 s
						d _A	8.19 J=9Hz
						d _B	7.58 J=9Hz
						d _C	6.94 J=9Hz
						d _D	6.91 J=9Hz
						d _E	6.97 J=16Hz
						d _F	7.23 J=16Hz
5i	271.5 (4.30) 362.5 (4.12)	1607 1585 1511	1668	987	2452	OCH ₂	4.03 t J= 6Hz
						SCH ₂	3.63 t J= 6Hz
						CVI H	7.49 d J= 7Hz
						CV H	6.53 d J= 7Hz
						d _A	8.21 J=9Hz
						d _B	7.59 J=9Hz
						d _C	6.96 J=9Hz
						d _D	6.91 J=9Hz
						d _E	6.94 J=16Hz
						d _F	7.23 J=16Hz
5j	271.0 (4.28) 360.5 (4.05)	1605 1590 1510	1655	958	2455	OCH ₂	4.00 t J= 6Hz
						SCH ₂	3.41 t J= 6Hz
						CVI H	6.49 s
						CV CH ₃	2.24 s
						d _A	8.21 J=9Hz
						d _B	7.59 J=9Hz
						d _C	6.94 J=9Hz
						d _D	6.91 J=9Hz
						d _E	6.94 J=16Hz
						d _F	7.22 J=16Hz

Table 4(continued)

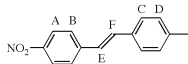
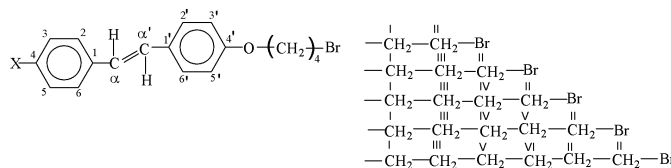
Comp.	UV/VIS	IR (cm ⁻¹)				¹ H NMR δ (ppm)	
	λ max (lg ε)	νC=C	νC=O	HC=CH	νSCH ₂		
			trans			5e-j	
5k	292.5 (4.40)	1604	1659	968	2401	OCH ₂ 4.11 t J= 6Hz	d _A 8.07 J=9Hz
	326.0 (4.46)	1592				SCH ₂ 3.38 t J= 6Hz	d _B 7.43 J=9Hz
		1511				CVI H 7.45 d J= 7Hz	d _C 7.16 J=9H
						CV H 6.63 d J= 7Hz	d _D 6.76 J=9Hz d _E 6.92 J=16Hz d _F 7.29 J=16Hz
5l	293.0 (4.37)	1604	1648	967	2669	OCH ₂ 4.03 t J= 6Hz	d _A 7.43 J=9Hz
	323.0 (4.41)	1575				SCH ₂ 3.26 t J= 6Hz	d _B 6.96 J=9Hz
		1511				CV H 6.37 s	d _C 6.90 J=9Hz
						CVI CH ₃ 2.46 s	d _D 6.63 J=9Hz d _E 7.00 J=16Hz d _F 7.64 J=16Hz

Table 5

¹³C NMR Shifts of **3a-3f**

Carbon	3a	3b	3c	3d	3e	3f
C-1	137.55	137.63	144.30	144.14	144.31	136.20
C-2,6	127.10	126.78	128.42	128.31	128.43	128.87
C-3,5	128.67	128.64	124.92	124.04	124.15	128.76
C-4	127.34	127.25	146.48	146.25	146.40	132.64
C- α	126.31	126.26	124.17	123.99	124.03	127.77
C- α'	128.02	126.16	132.90	132.76	132.92	125.14
C-1'	130.96	130.45	129.13	128.87	128.89	129.65
C-2',6'	127.81	127.75	128.42	126.38	126.48	127.36
C-3',5'	115.01	114.76	114.92	114.73	114.84	114.72
C-4'	157.79	158.43	159.29	159.35	159.68	158.98
C-I	67.94	65.39	68.87	66.94	67.66	67.76
C-II	28.97	29.91	29.83	29.47	28.93	29.02
C-III	-	32.34	32.48	33.46	33.56	32.62
C-IV	-	-	-	27.90	28.34	27.87
C-V	-	-	-	-	24.77	25.24
C-VI	-	-	-	-	-	33.71

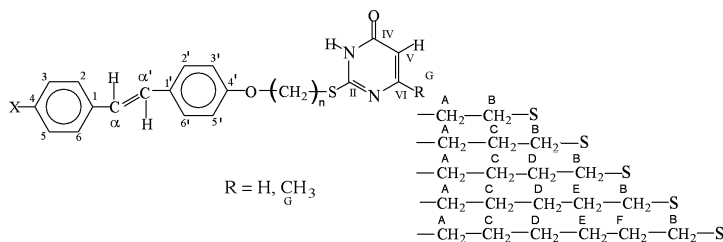
The fluorescence properties of compounds **5a-5l** have been investigated, the fluorescence emission maxima and quantum yields are summarized in Table 4. The (*E*)-2-stilbenyloxyalkylthiouracils (**5a,5c,5k**) and (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5l**) are moderately fluorescent, having emission bands at 370-380 nm with the main excitation peak at 320-360 nm at dioxane. The (*E*)-2-[4'-nitrostilbenyl-4-oxyalkylthio]-6-methyluracils (**5f,5h,5j**) are highly fluorescent, having emissions bands at 520 nm with the main excitation

peak at 360 nm in dioxane. Clearly, the substitution of (*E*)-stilbenylalkoxy group at the angular sulfur atom of 2-thiouracil (or 2-thio-6-methyluracil) serves as a useful method of converting these compounds into fluorescent derivatives.

Conclusions.

- It follows from the obtained results that the fluorescence of **5a-5l** can be attributed to the introduced fluorophore *i.e.* (*E*)-stilbenyl moiety.

Table 6
¹³C NMR Shifts of **5a-5f**



Carbon	5a	5b	5c	5d	5e	5f
C-1	136.06	137.50	137.44	137.31	146.18	146.49
C-2,6	126.32	126.41	127.35	126.29	128.34	126.51
C-3,5	128.14	128.69	127.81	129.72	124.46	124.16
C-4	127.61	127.37	127.35	128.34	146.12	146.30
C-α	126.23	126.34	126.20	126.64	136.50	132.97
C-α'	128.00	127.81	127.47	129.23	124.13	124.31
C-1'	128.65	128.74	128.71	129.83	129.53	128.57
C-2',6'	127.75	127.71	127.71	128.94	129.16	126.56
C-3',5'	114.16	114.75	114.88	115.75	114.81	115.04
C-4'	155.62	157.76	157.60	159.35	160.09	159.92
C-II	168.89	167.05	166.4	169.02	165.65	165.70
C-IV	161.18	160.63	161.56	161.70	160.65	164.56
C-V	108.67	108.86	108.85	109.30	109.11	108.42
C-VI	145.35	162.24	149.76	162.84	146.00	160.01
C _A	67.31	67.44	65.61	67.50	65.74	65.98
C _B	32.63	32.54	28.51	28.57	28.47	28.87
C _C	-	-	28.24	29.42	28.34	27.32
C _D	-	-	-	-	-	-
C _E	-	-	-	-	-	-
C _F	-	-	-	-	-	-
C _G	-	19.17	-	18.94	-	24.05

- (*E*)-4'-Nitrostilbenyl-4-oxyalkylthiouracils (**5e,5g,5i**) and (*E*)-4'-nitrostilbenyl-4-oxyalkylthio-6-methyluracils (**5f,5h,5j**) may well be the best fluorescent derivatives of uracil yet prepared.
- (*E*)-Configuration in the stilbene part of the molecules of **5a-5l** can be determined on the basis of the analysis of UV/VIS and IR spectra of these compounds.
- The presence of the 6-methyl substituent in the uracil ring of (*E*)-2-stilbenyloxyalkylthio-6-methyluracils (**5b,5d,5f,5h,5j,5l**) can be identified on the basis of analysis of ¹H NMR, (Table 4), ¹³C NMR (Table 6) and EI mass spectra (Tables 8 and 9) of these compounds.
- The differences in the ¹³C NMR spectra of **5a-5l** in the number and positions in the range 160-170 ppm of the C II, C IV and C VI carbon atoms of uracil ring, allow the differentiation of metamers **5b**, **5c** and **5f**, **5g** as well as **5h**, **5i**.
- The basic mass fragmentation of the molecular ion of **5a-5l** is due to cleavages of Csp³-O bonds of oxyalkylthio chains of (*E*)-stilbenyloxy-alkylthio-uracils.

- The inductive cleavage of these bonds produces even electron fragment ions **d**. The cleavage of these bonds according to McLafferty rearrangement produces odd-electron fragment ions **c** (Schemes 2,3; Tables 9, 10).
- The values of μ₁ and μ₂ (*i.e.* the ratio of intensities of **d** and **c** fragment ions peaks to those of the molecular ion peaks M⁺) depend on the structure of (*E*)-2-stilbenyloxyalkylthiouracils (**5c,5g,5i**) and (*E*)-stilbenyloxyalkylthio-6-methyluracils (**5b,5f,5h**). The differences in the values of μ₁ and μ₂ are useful for differentiation between metamers of these compounds derivatives of uracil (**5b,5f,5h**) and 6-methyluracil (**5c,5g,5i**).

EXPERIMENTAL

The purity of all described compounds was checked by m.p.'s, TLC and elemental analysis. Melting points (uncorrected) were determined on a Bötius microscope hot stage. R_f values refer to TLC silica gel F₂₅₄ TLC plates (Merck) developed with CHCl₃ - *n*-hexane 4:1 (**3a-3f**) and CHCl₃ - MeOH 5:1 (**5a-5l**) and observed under UV light (λ=254 and 366 nm). UV/VIS spectra were recorded with a Specord UV/VIS spectrophotometer in dioxane. IR spectra were recorded with a FT-

Table 7
¹³C NMR Shifts of **5g-5l**

Carbon	5g	5h	5i	5j	5k	5l
C-1	144.31	146.49	146.27	144.18	135.89	136.20
C-2,6	123.83	126.51	126.38	126.39	127.88	127.87
C-3,5	124.04	124.16	123.98	123.98	128.78	130.03
C-4	146.22	146.30	146.27	144.18	132.92	132.79
C-α	132.83	132.97	132.77	132.86	126.06	125.63
C-α'	124.15	124.16	124.05	124.07	128.50	128.84
C-1'	129.14	129.14	128.85	128.83	131.17	130.35
C-2',6'	126.47	128.41	124.31	128.32	127.41	127.44
C-3',5'	116.00	114.89	114.77	114.80	115.49	115.02
C-4'	160.00	159.41	159.41	159.92	157.38	158.60
C-II	161.70	165.70	165.65	165.40	163.91	157.15
C-IV	160.89	164.56	164.86	163.98	161.88	160.87
C-V	109.68	108.42	108.22	108.33	109.45	109.03
C-VI	146.18	160.30	146.0	160.92	145.52	165.09
C _A	67.72	65.48	67.26	67.73	69.22	68.16
C _B	31.27	28.47	30.24	30.57	31.65	31.08
C _C	28.56	28.34	28.17	28.86	28.59	28.82
C _D	-	-	25.87	25.20	25.04	25.21
C _E	-	-	-	28.75	27.93	28.56
C _F	-	-	-	-	27.67	27.99
C _G	-	24.05	-	24.23	-	21.54

Table 8
 Elemental Composition and Relative Intensities of the Ion Peaks in the spectra of
5a-5d and **5k-l** According to High Resolution Data

Ion	m/z	Elemental composition	% Relative intensity					
			5a	5b	5c	5d	5k	5l
M⁺ a	350	C ₂₀ H ₁₈ N ₂ O ₂ S	10	-	-	-	-	-
	364	C ₂₁ H ₂₀ N ₂ O ₂ S	-	7	18	-	-	-
	378	C ₂₂ H ₂₂ N ₂ O ₂ S	-	-	-	28	-	-
	440	C ₂₄ H ₂₅ N ₂ O ₂ SCl	-	-	-	-	72	-
	454	C ₂₅ H ₂₇ N ₂ O ₂ SCl	-	-	-	-	-	18
b	222	C ₁₆ H ₁₄ O	7	8	-	-	-	-
	236	C ₁₇ H ₁₆ O	-	-	3	6	-	-
	312	C ₂₀ H ₂₁ OCl	-	-	-	-	4	3
c	196	C ₁₄ H ₁₂ O	27	14	14	22	-	-
	230	C ₁₄ H ₁₁ OCl	-	-	-	-	77	100
d	155	C ₆ H ₇ N ₂ OS	100	-	-	-	-	-
	169	C ₇ H ₉ N ₂ OS	-	100	100	-	-	-
	183	C ₈ H ₁₁ N ₂ OS	-	-	-	100	-	-
	211	C ₁₀ H ₁₅ N ₂ OS	-	-	-	-	100	-
	225	C ₁₁ H ₁₇ N ₂ OS	-	-	-	-	-	27
e	95	C ₄ H ₃ N ₂ O	13	-	7	-	13	-
	109	C ₅ H ₅ N ₂ O	-	11	-	6	-	5
f	70	C ₃ H ₄ NO	8	-	7	-	20	-
	84	C ₄ H ₆ NO	-	9	-	10	-	25

IR Bruker IFS – 113 v spectrophotometer in KBr pellets. The ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) spectra were recorded on a Varian Mercury Spectrometer operating at 300.07 MHz (proton) or 75.46 MHz (carbon). Data were obtained from CDCl₃ solutions. The chemical shifts were referenced to tetramethylsilane. Chemical shifts are given in the δ scale (ppm) and coupling constants in Hertz. ¹H NMR (300.07) spectra were recorded with spectral width 9 KHz, acquisition time 2.0 s, pulse width 6 μs and double precision acquisition. ¹³C NMR (75.460 MHz) spectra were recorded with spectral width 18.76 KHz, acquisition time 1.0 s, recycle delay 1.0 s

and pulse width 15 μs. Heteronuclear 2D ¹³C NMR- ¹H NMR chemical shift correlation experiments were carried out using HETCOR spectra. The spectra were acquired with 2K data points, 256 increments and spectral width 19.63 KHz for ¹³C and 4.97 KHz for ¹H.

Elemental analyses were performed with a Vector Euro EA 3000 analyzer. Low- and high-resolution mass spectra were recorded on an AMD - Intectra GmbH-Harpstedt D-27243 Model 402 two - sector mass spectrometer (ionizing voltage 70 eV, accelerating voltage 8 kV, resolution 10,000). Samples were introduced by a direct insertion probe at the source temperature

Table 9

Elemental Composition and Relative Intensities of the Ion Peaks in the Spectra of **5e-5j** According to High Resolution Data

Ion	m/z	Elemental composition	% Relative intensity					
			5e	5f	5g	5h	5i	5j
M⁺ a	409	C ₂₁ H ₁₉ N ₃ O ₄ S	3	-	-	-	-	-
	423	C ₂₂ H ₂₁ N ₃ O ₄ S	-	6	16	-	-	-
	437	C ₂₃ H ₂₃ N ₃ O ₄ S	-	-	-	4	27	-
	451	C ₂₄ H ₂₅ N ₃ O ₄ S	-	-	-	-	-	20
b	211	C ₁₃ H ₉ NO ₂	37	11	9	7	14	7
c	241	C ₁₄ H ₁₁ NO ₃	30	12	14	9	45	26
d	169	C ₇ H ₉ N ₂ OS	100	-	-	-	-	-
	183	C ₈ H ₁₁ N ₂ OS	-	100	100	-	-	-
	197	C ₉ H ₁₃ N ₂ OS	-	-	-	100	100	-
	211	C ₁₀ H ₁₅ N ₂ OS	-	-	-	-	-	93
e	165	C ₁₃ H ₉	59	25	22	19	30	24
f	152	C ₁₂ H ₈	11	11	6	3	8	7
g	109	C ₅ H ₅ N ₂ O	-	12	-	16	-	40
	95	C ₄ H ₃ N ₂ O	13	-	35	-	25	-

Table 10

Values of μ Calculated from the EI Mass Spectra of Metameric **5b, 5c, 5f, 5g, 5h, 5i**

Comp	5b	5c	5f	5g	5h	5i
μ_1	0.7	1.8	0.6	1.6	0.4	2.7
μ_2	2.0	0.7	2.0	0.8	2.25	1.66

Table 11

UV/VIS and Fluorescence Spectra of **5a-5l**

Comp.	UV/VIS λ_{max} [nm], (log ϵ) (dioxane)	Fluorescence		Quantum yields [ϕ]
		Excitation wave [nm] (dioxane)	Emission wave [nm] (dioxane)	
5a	303.0 (4.53)	320	370	0.01
	320.0 (4.49)			
5b	304.5 (4.51)	320	370	0.01
	320.0 (4.49)			
5c	303.5 (4.53)	320	380	0.01
	320.0 (4.49)			
5d	304.5 (4.50)	320	370	0.01
	320.5 (4.48)			
5e	265.0 (4.32)	360	520	0.002
	359.0 (4.17)			
5f	266.0 (4.32)	360	530	0.002
	359.0 (3.72)			
5g	271.0 (4.27)	360	520	0.002
	261.0 (4.08)			
5h	270.0 (4.29)	360	520	0.003
	259.5 (4.09)			
5i	271.9 (4.30)	360	520	0.002
	362.5 (4.12)			
5j	271.9 (4.28)	360	520	0.003
	360.5 (4.05)			
5k	308.0 (4.50)	326	380	0.01
	326.5 (4.50)			
5l	308.5 (4.53)	326	380	0.01
	326.0 (4.54)			

of ~ 150 °C. The elemental composition of the ions were determined by a peak matching method relative to perfluorokerosene and using the same instrument. All masses measured were in agreement with those of the composition given in column 3 of Tables 8 and 9 to within ± 2 ppm. The B/E linked scan spectra in the first field - free region were investigated using helium as the collision gas at a pressure of 1.73×10^{-5} with the ion source temperature of 180 °C, ionization energy of 70 eV and an accelerating voltage of 8 kV. The values of μ_1 and μ_2 were calculated as averages of three measurements. Fluorescence corrected spectra were taken in dioxane (isocratic grade for liquid chromatography-Merck) with a Perkin Elmer MPF-3 instrument. Fluorescence quantum yields (in the same solvent) were calculated on the basis of the value 0.55 for quinine sulfate. (*E*)-Stilbenol-4, (*E*)-4'-nitrostilbenol-4 and *E*-4'-chlorostilbenol-4 were obtained according to the literature [38].

General Procedure for the Preparation of (*E*)-Bromoalkoxystilbenes (**3a-3f**).

A 20 ml solution of (*E*)-stilbenol-4, (2 mmole) or (*E*)-4'-nitrostilbenol-4, (*E*)-4'-chloro-stilbenol-4) in of 0.3 *M* NaOH in DMSO was stirred at room temperature while 4 mmole of dibromoalkanes (1,2-dibromoethane; 1,3-dibromopropane; 1,4-dibromobutane; 1,5-dibromopentane; 1,6-dibromohexane) was added dropwise. After stirring for 3 hours precipitated solid was filtered. The filtrate was then acidified (pH 3) with 6 *M* HCl and diluted with 80 ml of cold water. The precipitated solid was collected by filtration, washed with water and dried at room temperature under vacuum. Recrystallization from ethanol afforded compounds **3a-3f**.

General Procedure for the Preparation of (*E*)-2-Stilbenyloxyalkylthiouracils (**5a-5l**).

2-Thiouracil (or 2-thio-6-methyluracil) (0.07 mole) in 10 ml of 0.3 *M* methanolic NaOH was stirred in room temperature while 0.07 mole of corresponding (*E*)-bromoalkoxystilbene (**3a-3f**) in 10 ml of acetone solution was added dropwise. The reaction mixture was stirred at room temperature for 8 hours. The obtained solid was filtered, the filtrate was acidified (pH 3) with 6 *M* HCl and diluted with 70 ml of cold water. The precipitated solid was collected by filtration, washed with water and dried at room temperature under vacuum. Recrystallization from ethanol afforded compounds **5a-5l**.

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