Synthesis and Physicochemical Properties of New Fluorescent Derivatives of Cytosine

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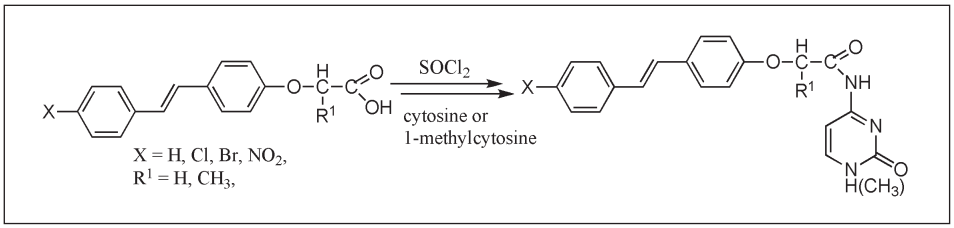

Sixteen new fluorescent N^4-(*E*)-stilbenyloxyalkylcarbonyl-cytosines **9**-**16** and N^4-(*E*)-stilbenyloxyalkylcarbonyl-1-methylcytosines **17**-**24** have been synthesized. The differences in ^{1}H and ^{13}C NMR spectra in two solvents (DMSO and TFA) have been pointed out and discussed. Assignment of the signals in the spectra of the compounds **9**-**24** in NMR in DMSO-d_6 solutions has been made the basis of the homonuclear (COSY) and heteronuclear (HETCOR) spectra. The effect of the substituent (Cl, Br, NO_2) on the stilbene moiety on the fluorescence spectrum of each compound has been discussed.

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Introduction.

The modification of nucleobases or deoxyribose moiety in DNA by (*E*)-stilbene containing derivatives has been widely reported in literature [1-5] because of their fluorescent properties and high quantum yield. These compounds are potentially useful as probes in the study of the structure and dynamics of nucleic acid and their complexes with proteins; they may also find use as fluorescent labels for nucleic-acid-based biomedical diagnostic methods.

On the other hand, there is a wealth of published information on the fluorescent compounds of cytosine and cytidine [6-10], including their N-acyl derivatives [11]. This fact has stimulated the preparation of a series of N^4-(*E*)-stilbenyloxyalkylcarbonyl-cytosines (**9-16**) and N^4-(*E*)-stilbenyloxyalkylcarbonyl-1-methylcytosines (**17-24**) aiming at obtaining specific fluorescent modification of these compounds. The fluorescent moieties chosen for the study are (*E*)-stilbene, (*E*)-4'-chlorostilbene, (*E*)-4'-bromostilbene and (*E*)-4'-nitrostilbene.

This paper deals with the synthesis and physicochemical properties of compounds **9-24**. The NMR spectra have been obtained in two solvents to examine the influence of acid solvent (TFA) on the changes in ^{1}H and ^{13}C NMR spectra relative to those measured in standard DMSO-d_6 solutions. The spectral analysis (IR, UV/VIS and ^{1}H NMR) has been also performed to check the possibility of differentiation of metamers existing in those series of compounds.

Investigation of the fluorescence spectra has been undertaken to check the dependence of quantum yields and emission maxima on the substituents (H, Cl, Br, NO_2) in the (*E*)-stilbene moiety.

Results and Discussion.

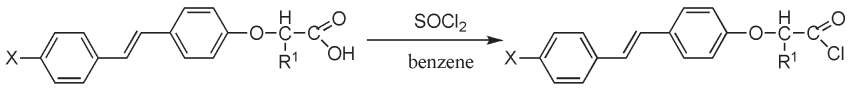
A series of sixteen new fluorescent N^4-[(*E*)-stilbenyloxyalkylcarbonyl-cytosines (**9-16**) and N^4-(*E*)-stilbenyloxyalkylcarbonyl-1-methylcytosines (**17-24**) have been synthesized in the reaction of the corresponding (*E*)-4-chlorocarbonylalkoxystilbenes (**1-8**) with cytosine (or 1-methylcytosine) in boiling pyridine solutions, in the presence of a small amount of DMAP (Scheme 1). Noteworthy is the fact that the presence of the methyl substituent at the annular nitrogen atom N-1 of the pyrimidine ring of molecules (**17-24**) changes the physicochemical properties of these compounds relative to those of **9-16**. In particular the values of the melting points of **17-24** are lower and the solubility in organic solvents is higher. This fact allows making use of column chromatography for purification of **17**, **18**, **20**, **22**, **23**, **24**.

The structures of all compounds obtained were determined by examining their UV/VIS, IR, ^{1}H and ^{13}C NMR spectra as well as on the basis of elemental analyses (Tables I-Vd).

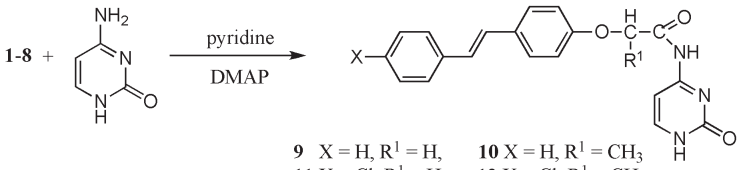
This paper also presents the physicochemical properties of four stilbenes (**4**, **6**, **7**, **8**) unknown in literature (Table II). They have been synthesized according to literature procedure with some modifications in the preparation and isolation procedures. The physicochemical properties of (*E*)-4-chlorocarbonylalkoxystilbenes (**1**, **2**, **3**, and **5**) have been determined by us earlier [12].

The data from ^{1}H and ^{13}C NMR spectra of compounds **9-24** recorded in DMSO-d_6 and CF_3COOD solutions are given in Tables III and IVa-d. Assignments of the ^{1}H and ^{13}C NMR resonances of these compounds were deduced on the basis of signal multiplicities, by comparison with literature data [13-19] and by the concerted application

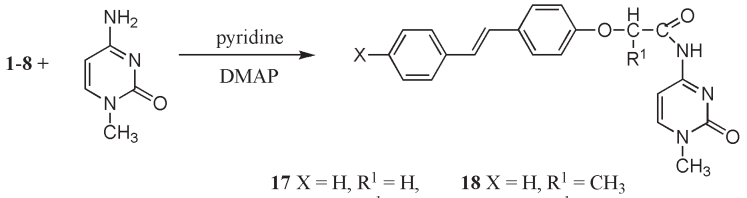
Scheme 1



1 X = H, R^1 = H **2** X = H, R^1 = CH_3
3 X = Cl, R^1 = H **4** X = Cl, R^1 = CH_3
5 X = Br, R^1 = H **6** X = Br, R^1 = CH_3
7 X = NO_2, R^1 = H **8** X = NO_2, R^1 = CH_3



9 X = H, R^1 = H, **10** X = H, R^1 = CH_3
11 X = Cl, R^1 = H **12** X = Cl, R^1 = CH_3
13 X = Br, R^1 = H **14** X = Br, R^1 = CH_3
15 X = NO_2, R^1 = H **16** X = NO_2, R^1 = CH_3



17 X = H, R^1 = H, **18** X = H, R^1 = CH_3
19 X = Cl, R^1 = H **20** X = Cl, R^1 = CH_3
21 X = Br, R^1 = H **22** X = Br, R^1 = CH_3
23 X = NO_2, R^1 = H **24** X = NO_2, R^1 = CH_3

Table Ia

Chemical and Physical Data of Compounds **9-16**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield [%]	R_f [a]	Elemental analysis, Calculated (Found) C	H	N
9	$C_{20}H_{17}N_3O_3$ 347 x $1H_2O$	286-289	31	0.70	65.75 (65.93)	5.21 (5.26)	11.51 (11.16)
10	$C_{21}H_{19}N_3O_3$ 361 x 0,5 H_2O	265-268	33	0.78	68.11 (68.15)	5.41 (5.48)	11.35 (11.58)
11	$C_{20}H_{16}N_3O_3Cl$ 381.5	305-310	39	0.65	62.91 (62.56)	4.19 (4.55)	10.01 (10.39)
12	$C_{21}H_{18}N_3O_3Cl$ 395.5	296-301	35	0.67	63.79 (63.80)	4.56 (4.65)	10.63 (10.54)
13	$C_{20}H_{16}N_3O_3Br$ 426 x 0,5 H_2O	314-316	40	0.58	55.17 (55.38)	3.91 (3.99)	9.66 (9.63)
14	$C_{21}H_{18}N_3O_3Br$ 440	300-305	30	0.74	57.27 (57.10)	4.09 (4.45)	9.55 (9.61)
15	$C_{20}H_{16}N_4O_5$ 392 x 1 H_2O	315-319	39	0.56	58.54 (58.90)	4.39 (4.51)	13.66 (13.62)
16	$C_{21}H_{18}N_4O_5$ 406 x 1 H_2O	308-311	40	0.64	59.43 (59.96)	4.71 (4.98)	13.21 (12.99)

[a]- $CHCl_3$:CH_3OH 5:1.

involvement of the two dimensional NMR techniques HETCOR and COSY, which clearly showed the connectivity of hydrogen and carbon atoms involved. The employment of these techniques allowed unequivocal assignment of the spectra especially for the stilbene rings.

The (*E*)-configuration in the stilbene moiety was determined on the basis of their UV/VIS and IR spectra. According to literature [20-22] (*E*)-stilbenes exhibit 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.

Table Ib

Chemical and Physical Data of Compounds **17-24**

Comp.	Formula (mol. weight)	M.p. [°C]	Yield [%]	R_f [a]	Elemental analysis, Calculated (Found) C	H	N
17	$C_{21}H_{19}N_3O_3$ 361	261-263	42	0.63	69.81 (69.49)	5.26 (4.97)	11.63 (11.64)
18	$C_{22}H_{21}N_3O_3$ 375	115-120	51	0.75	70.40 (70.39)	5.60 (5.24)	11.20 (11.05)
19	$C_{21}H_{18}N_3O_3Cl$ 395.5	261-267	40	0.67	63.72 (63.52)	4.55 (4.50)	10.62 (10.25)
20	$C_{22}H_{20}N_3O_3Cl$ 409.5	210-215	62	0.75	64.47 (64.21)	4.88 (4.90)	10.26 (9.98)
21	$C_{21}H_{18}N_3O_3Br$ 440	267-273	47	0.65	57.27 (56.99)	4.09 (4.05)	9.55 (9.34)
22	$C_{22}H_{20}N_3O_3Br$ 454	215-220	52	0.81	58.15 (58.06)	4.41 (4.26)	9.25 (9.18)
23	$C_{21}H_{18}N_4O_5$ 406	300-303	72	0.67	62.07 (61.88)	4.43 (4.21)	13.79 (13.54)
24	$C_{22}H_{20}N_4O_5$ 420	225-230	85	0.71	62.86 (62.64)	4.76 (4.59)	13.33 (13.11)

Table II

Chemical and Physical Data of Compounds **4**, **6**, **7**, **8**

Comp.	*X*	R^1	Formula (mol. weight)	M.p. [°C]	Yield [%]	Elemental analysis, Calculated (Found) C	H	N
4	Cl	CH_3	$C_{17}H_{14}O_2Cl_2$ 321	68	98	63.55 (63.79)	4.36 (4.35)	-
6	Br	CH_3	$C_{17}H_{14}O_2BrCl$ 365.5	75	97	55.81 (55.88)	3.83 (3.85)	-
7	NO_2	H	$C_{16}H_{12}NO_4Cl$ 317.5	109	95	60.47 (60.55)	3.78 (3.77)	4.41 (4.30)
8	NO_2	CH_3	$C_{17}H_{14}NO_4Cl$ 331.5	54	99	61.54 (61.59)	4.22 (4.23)	4.22 (4.01)

Table III

IR Data of **9-24**

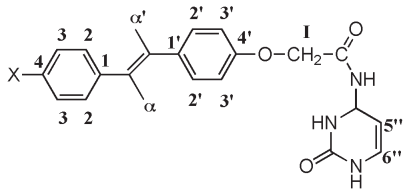
Compd.	IR (cm^{-1}) νC=O I amide	νC=O in pyrimid.ring	Arom.skeletal vibrations	δ CH=CH (trans)	Ar-NO_2
9	1750, 1742	1693	1606, 1510	961	-
10	1709	1699	1607, 1509	965	-
11	1750, 1744	1694	1606, 1510	964	-
12	1706	1699	1606, 1510	966	-
13	1750, 1742	1693	1605, 1511	962	-
14	1720	1696	1609, 1498	962	-
15	1749	1698	1609, 1510	963	1339
16	1749	1696	1605, 1510	967	1337
17	1728	1669	1604, 1510	968	-
18	1722	1669	1604, 1510	964	-
19	1728	1671	1607, 1511	960	-
20	1716, 1699	1668	1603, 1498	970	-
21	1729	1663	1604, 1509	966	-
22	1717, 1699	1668	1603, 1499	970	-
23	1724	1654	1606, 1510	965	1338
24	1709	1653	1604, 1508	967	1338

The IR spectra, in the range 1700-1751 cm^{-1} show intense bands corresponding to carbonyl stretching vibrations (ν C=O). This band can be assigned to the acyclic amide whereas the vibration in the 1663-1699 cm^{-1} area can be assigned to ν C=O (mostly) in pyrimidine ring. The IR spectra confirm also the (*E*)-configuration in stilbene moiety revealing the bond attributed to the out-of-plane deformation vibration of the C-H bond of the (*E*)-ethylene bridge [23,24].

The ^1H NMR spectra are recorded in a standard DMSO-d_6 solution (**9**, **10**, **11**, **12**, **17**, **18**, **20**, **22**, **24**) and CF_3COOD solution (**13**, **14**, **15**, **16**, **19**, **21**, **23**) because of low solubility of the latter in practically all organic solvents. The NMR spectra of compounds **9** and **24** are measured in both DMSO-d_6 and TFA solutions for the sake of comparison. The data assignment for these compounds was based on the examination of two-dimensional HETCOR and COSY spectra for **9**, **20** and **22** (Tables IVa-IVd). The methylation of N-1 atom in pyrimidine ring has no important influence on hydrogen shifts in all investigated compounds, exept for H-6" which is shifted downfield by about 0.3 ppm.

Protonation of the molecules by CF_3COOD, in general, causes a shift of the resonances, particularly of 5"-H atom and 6"-H atom in cytosine moiety. The δ values of the 5"-H in the

Table IVa
^{1}H NMR Data of **9, 11, 15**

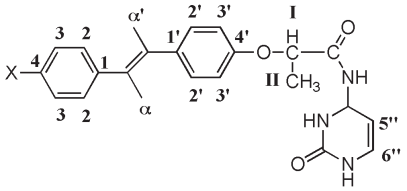


9 X=H, **11** X=Cl, **15** X=NO_2

Comp.	I -CH_2-	2	3	4	2'	3'	α	α'	5"	6"
9	4.85s	7.55d J=7	7.36t J=7	7.24t J=7	7.51d J=9	6.94d J=9	7.11d J=16	7.09d J=16	7.03d J=7	7.86d J=7
9[a]	4.99s	7.51d J=7	7.35t J=7	7.26t J=7	7.55d J=9	6.95d J=9	7.12d J=16	7.05d J=16	6.95d J=7	8.25d J=7
11	4.85s	7.59d J=9	7.42d J=9	-	7.55d J=9	6.95d J=9	7.24d J=16	7.11d J=16	7.03d J=7	7.81d J=7
15[a]	4.99s	7.69d J=9	8.27d J=9	-	7.59d J=9	7.02d J=9	7.13d J=16	7.31d J=16	7.01d J=7	8.53d J=7

[a]-in TFA

Table IVb
^{1}H NMR Data of **10.12,14, 16**



10 X=H, **12** X=Cl, **14** X=Br, **16** X=NO_2

Comp.	I -CH-	II -CH_3	2	3	4	2'	3'	α	α'	5"	6"
10	5.08q J=7	1.53d J=7	7.56d J=7	7.36t J=7	7.24t J=7	7.55d J=9	6.94d J=9	7.19d J=16	7.10d J=16	7.07d J=7	7.87d J=7
12	5.07q J=7	1.52d J=7	7.56d J=9	7.41d J=9	-	7.54d J=9	6.90d J=9	7.21d J=16	7.09d J=16	7.05d J=7	7.86d J=7
14[a]	5.13q J=7	1.76d J=7	7.46d J=9	7.37d J=9	-	7.54d J=9	6.99d J=9	7.09d J=16	7.01d J=16	6.94d J=7	8.48d J=7
16[a]	5.18q J=7	1.76d J=7	7.67d J=9	8.23d J=9	-	7.55d J=9	7.01d J=9	7.09d J=16	7.28d J=16	6.98d J=7	8.49d J=7

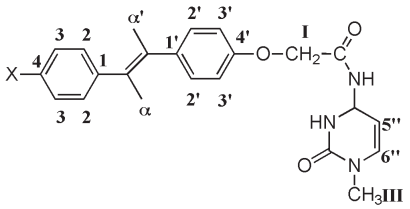
[a]-in TFA

pyrimidine ring was observed from 7.03 to 7.07 ppm (**9-16**), and from 7.08 to 7.14 ppm (**17-24**) in DMSO solutions, and were upfield shifted in TFA solutions by about 0.2 ppm. The replacement of DMSO with TFA solvent produces the most significant changes in 6"-H assignments, for all compounds investigated. The differences in δ values for the appropriate protons in different solvents are the same. Noteworthy is the fact that the presence of CH_3 substituent at the N-1 atom of pyrimidine ring does not influence this value. The signals assigned to these protons are in the range 7.8-7.9 ppm (**9-12**) and 8.12-8.18 ppm (**17**, **19**, **20**, **22**, **24**) in DMSO solutions and are downfield shifted by more than 0.4 ppm in TFA solutions. For the sake of comparison, the ^{1}H NMR spectrum of **9** in DMSO shows a doublet at 7.86 ppm and at 8.25 ppm in TFA due to 6" proton. Compound **24** exhibits a doublet at δ 8.13 in DMSO and at δ 8.51 in TFA, assignable to this 6" proton.

The ^{13}C NMR spectra have also been obtained in two solvents, DMSO-d_6 and deuterated TFA. Assignments of the ^{13}C NMR resonances data are given in Tables Va-Vd. The presence of CH_3 group at the N-1 site in 1-methylcytosine derivatives caused a shift of the resonance of C-6" atom in pyrimidine ring to higher δ values, and of C-V atom of these compounds to lower δ values than those of unsubstituted cytosine ring derivatives. This fact allows a differentiation between metameric **10** and **17** ($C_{21}H_{19}N_3O_3$), **12** and **19** ($C_{21}H_{18}N_3O_3Cl$), as well as **16**

Table IVc

^{1}H NMR Data of **17, 19, 23**



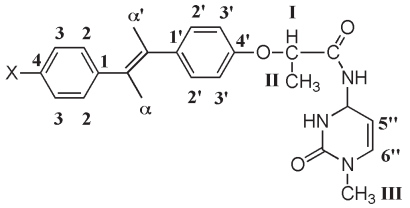
17 X=H, **19** X=Cl, **23** X=NO_2

Comp.	I -CH_2-	III -CH_3	2	3	4	2'	3'	α	α'	5"	6"
17	4.85s	3.22s	7.57d J=7	7.36t J=7	7.25t J=7	7.56d J=9	6.95d J=9	7.21d J=16	7.11d J=16	7.08d J=7	8.13d J=7
19[a]	4.85s	3.88s	7.59d J=9	7.48d J=9	-	7.56d J=9	6.95d J=9	7.23d J=16	7.11d J=16	7.06d J=7	8.12d J=7
23[a]	4.97s	3.87s	7.62d J=9	8.23d J=9	-	7.55d J=9	7.00d J=9	7.09d J=16	7.28d J=16	6.96d J=7	8.55d J=7

[a]-in TFA

Table IVd

^{1}H NMR Data of **18, 20, 22, 24**



18 X=H, **20** X=Cl, **22** X=Br, **24** X=NO_2

Comp	I -CH-	II -CH_3	III -CH_3	2	3	4	2'	3'	α	α'	5"	6"
18	5.32q J=7	1.53d J=7	3.19s	7.55d J=7	7.36t J=7	7.24t J=7	7.54d J=9	6.90d J=9	7.19d J=16	7.14 d J=16	7.09 d J=7	8.12d J=7
20	5.13q J=7	1.58d J=7	3.20s	7.63d J=9	7.46d J=9	-	7.59d J=9	6.96d J=9	7.27d J=16	7.15d J=16	7.14d J=7	8.18d J=7
22	5.13q J=7	1.58d J=7	3.21s	7.66d J=9	7.59d J=9	-	7.59d J=9	6.86d J=9	7.28d J=16	7.14d J=16	7.14d J=7	8.18d J=7
24	5.05q J=7	1.53d J=7	3.23s	7.81d J=9	8.21d J=9	-	7.63d J=9	6.94d J=9	7.27d J=16	7.48d J=16	7.08d J=7	8.13d J=7
24[a]	5.16q J=7	1.76d J=7	3.85s	7.65d J=9	8.19d J=9	-	7.52d J=9	6.99d J=9	7.07d J=16	7.25d J=16	6.94d J=7	8.51d J=7

[a]-in TFA

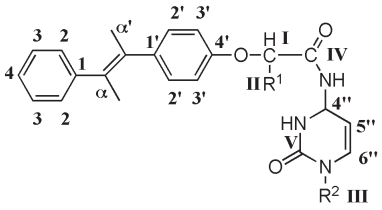
and **3** ($C_{21}H_{19}N_4O_5$). The data of appropriate C signals are as follows: **10** N^4-(E)-stilbenyloxyalkylcarbonyl-cytosine, C-6"-147.7 ppm, C-V-162.9 ppm; **17** N^4-(E)-stilbenyloxyalkylcarbonyl-1-methylcytosine, C-6"-151.3 ppm, C-V-161.9 ppm, C-III-37.6 ppm; **12** N^4-(E)-4'-chlorostilbenyloxyalkylcarbonyl-cytosine, C-6"-147.7 ppm, C-V-162.9 ppm; **19**- N^4-(E)-4'-chlorostilbenyloxyalkylcarbonyl-1-methylcytosine, C-6"-151.1 ppm, C-V 161.8 ppm, C-III-37.5 ppm; **16**- N^4-(E)-4'-nitrostilbenyloxyalkylcarbonyl-cytosine, C-6"-153.6 ppm; **23**- N^4-(E)-4'-nitrostilbenyloxyalkylcarbonyl-1-methylcytosine, C-6"-158.3 ppm, C-III-39.3 ppm.

The solvent replacement by a more acidic TFA caused changes in the ^{13}C NMR resonance signals, particularly of carbonyl and pyrimidine ring carbons. The most important changes appeared in the C-6" carbon resonances. In DMSO solutions these signals were observed in the range 147.5-147.8 ppm (for cytosine derivatives **9-16**) and 151.1-151.5 ppm (for 1-methylcytosine derivatives **17-24**) and in TFA solutions they were shifted by 6 and 7 ppm (respectively) to lower-field.

The resonances of IV C atoms (carbonyl group), in DMSO fall in the range 168.8-169.0 ppm (**9**, **11**, **17**, **19**) and 171.8-172.9 ppm (**10**, **12**, **18**, **20**, **22**, **24**), and were

Table Va

^{13}C NMR Shifts of **9, 10, 17, 18**

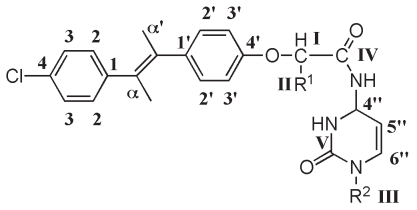


Carbon	**9**	**9**[a]	**10**	**17**	**18**
1	138.3	137.0	137.3	137.3	137.3
2	126.2	126.0	126.2	126.2	126.1
3	128.7	128.4	128.6	128.7	128.5
4	127.2	127.5	127.3	127.3	127.1
α	127.9	127.8	127.9	127.9	127.7
α'	126.5	126.8	126.6	126.5	126.4
1'	130.3	129.9	130.4	130.3	130.2
2'	127.8	127.7	127.8	127.8	127.6
3'	114.7	114.7	115.0	114.7	114.9
4'	157.4	155.4	156.7	157.4	156.5
4''	155.9	159.2	155.9	155.6	155.4
5''	94.6	96.0	94.6	94.8	94.7
6''	147.5	153.8	147.7	151.4	151.2
I	66.7	67.4	72.8	66.6	72.7
II	-	-	18.3	-	18.4
III	-	-	-	37.6	37.6
IV	169.0	173.5	172.2	168.9	172.9
V	162.8	-[b]	162.9	161.9	161.8

[a]-in TFA; [b]-hindered upon TFA signal.

Table Vb

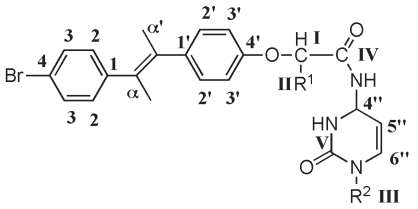
^{13}C NMR Shifts of **11, 12, 19, 20**



Carbon	**11**	**12**	**19**	**20**
1	136.3	136.3	136.3	136.3
2	127.9	127.9	127.9	128.0
3	128.7	128.6	128.6	128.7
4	131.5	131.5	131.4	131.5
α	128.8	128.7	128.8	128.7
α'	125.2	125.2	125.1	126.3
1'	130.1	130.1	130.1	130.2
2'	127.9	127.8	127.8	127.8
3'	114.8	115.1	114.8	115.1
4'	157.6	156.9	157.5	156.9
4''	156.0	155.9	155.6	155.6
5''	94.6	94.6	94.7	94.8
6''	147.6	147.7	151.1	151.5
I	66.7	72.8	66.7	72.7
II	-	18.3	-	18.3
III	-	-	37.5	37.6
IV	169.0	172.2	168.8	172.1
V	162.8	162.9	161.8	161.9

Table Vc

^{13}C NMR Shifts of **14** and **22**

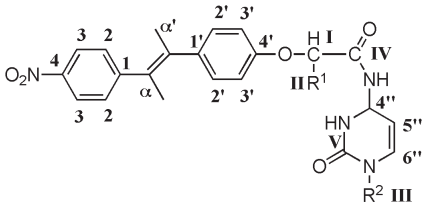


Carbon	**14**[a]	**22**
1	136.1	136.6
2	127.2	128.0
3	133.6	131.6
4	120.9	120.1
α	127.9	128.8
α'	127.4	125.3
1'	131.4	130.1
2'	127.4	128.2
3'	113.1	115.1
4'	154.8	156.9
4''	159.6	155.7
5''	96.1	94.8
6''	153.6	151.5
I	75.4	72.7
II	15.9	18.3
III	-	37.6
IV	177.2	172.1
V	-[b]	162.0

[a]-in TFA; [b]-hindered upon TFA signal.

Table Vd

^{13}C NMR Shifts of **15**, **16**, **24**



Carbon	**15** [a]	**16**[a]	**23**[a]	**24**[a]	**24**
1	145.9	145.5	146.2	145.9	144.2
2	128.9	128.6	129.1	128.9	128.6
3	125.8	125.4	125.9	125.8	124.3
4	145.9	145.6	146.2	145.9	145.7
α	132.8	132.3	133.0	132.7	132.6
α'	124.5	124.1	124.7	124.5	123.9
1'	132.5	132.2	132.7	132.6	129.5
2'	126.9	126.5	127.2	126.9	126.8
3'	115.2	116.0	115.4	115.9	115.0
4'	156.8	155.7	157.0	156.1	157.3
4''	159.7	159.7	158.5	158.6	155.4
5''	96.5	96.3	96.3	96.2	94.7
6''	151.3	153.6	158.3	157.8	151.3
I	67.4	75.1	67.5	75.4	72.7
II	-	15.7	-	16.1	18.4
III	-	-	39.3	39.0	37.6
IV	173.8	176.7	174.1	177.4	171.8
V	-[b]	-[b]	-[b]	-[b]	161.8

[a]-in TFA; [b]-hindered upon TFA signal.

Table VI
UV/VIS and Fluorescence Spectra of **9**-**24**

Comp.	UV/VIS λ_{max}	log ε [nm]	Excitation wave [nm]	Fluorescence emission wave [nm]	Quantum Yield Φ
9	320, 306	4.47, 4.45	350	385	0.01
10	320, 309	4.46, 4.38	350	385	0.02
11	324, 309	4.46, 4.45	350	383	0.10
12	323, 309	4.45, 4.34	350	383	0.10
13	326, 311	4.37, 4.35	350	388	0.02
14	326, 311	4.48, 4.45	350	388	0.01
15	381	4.29	410	605	0.38
16	379	4.28	410	605	0.49
17	321, 305	4.46, 4.45	350	387	0.01
18	321, 306	4.46, 4.39	350	387	0.02
19	324, 309	4.45, 4.44	350	385	0.01
20	324, 309	4.46, 4.46	350	385	0.01
21	324, 310	4.39, 4.37	350	388	0,02
22	323, 310	4.46, 4.45	350	388	0.01
23	379	4.33	410	605	0.50
24	379	4.33	410	605	0.53

shifted about 5 ppm to lower-field in TFA solutions. The upfield shifts of the C=O carbonyl signals of IV C atoms (**9**, **11**, **17**, **19**) are caused by the absence of $-CH_3$ (II) group in the alkyl moiety of these derivatives. The different values of C-4" signals are observed in DMSO and in TFA. They fall in the same range 155.4-156.0 ppm, both for cytosine and 1-methylcytosine derivatives and are shifted downfield 4 ppm in TFA solutions. The measured values of C-5" shifts were observed at 94.6-94.8 ppm in DMSO and were shifted downfield only by about 1.5 ppm in acid solvent.

It should be pointed out that the differences in the values of the chemical shifts in different solvents for appropriate carbon atoms are the same for both cytosine and 1-methylcytosine derivatives. This suggests a strong interaction between acidic solvent (TFA) and the compounds that are diluted in it and demonstrates that protonation occurs in TFA solution predominantly at the nitrogen in amide group.

The fluorescence properties of compounds **9**-**24** have been also investigated. The characteristic values in UV/VIS spectra, and the fluorescent emission maxima and quantum yields are summarized in Table VI. All spectra were recorded in DMF solution. Excitation spectra were also measured at the emission maxima, and the spectra were identical to the absorption curves for all investigated compounds. As we can see in Table VI, compounds **9**-**14**, and **17**-**22** show medium fluorescence quantum yields, but compounds **15**, **16** and **23**, **24** are strongly fluorescent. These compounds reveal also unexpectedly great difference between absorption maxima and fluorescent emission maxima. This fact predestinates these derivatives of cytosine to further photochemical investigations. The fluorescence spectra of substituted cytosine **9**-**14** and 1-methylcytosine **17**-**22** are similar, with almost the same value of fluorescence emission maxima. The type of substituent in the stilbene moiety affects the fluorescent properties of the compounds synthesized. The most suitable substituent is the $-NO_2$ group (**15**, **16** and **23**, **24**).

Conclusions.

The fluorescent data for **15**, **16** and **23**, **24** show that these cytosine derivatives are the best fluorescence compounds in our series, they show very high quantum yields.

Excitation of these compounds is possible at 410 nm, well outside the range of absorption of proteins and nucleic acids and indicates that these cytosine derivatives may prove suitable for nucleic-acid-based research.

The use of HETCOR and COSY NMR methods allows correct assignments of the resonance signals in the ^1H and especially in ^{13}C NMR spectra of the stilbene moiety.

The differences in the ^{13}C NMR and ^1H NMR spectra in two solvents (DMSO and TFA) permit assessment of the influence of protonation of the nitrogen atom on the chemical shifts.

Analysis of the ^1H NMR, UV/VIS and IR spectra has confirmed the (*E*)-configuration in the stilbene parts of the molecules of **9-24**.

EXPERIMENTAL

Purity of all compounds studied was checked by m.p.'s, TLC and elemental analysis. Melting points (uncorrected) were determined on a Böetius microscope hot stage. R_f values refer to TLC silica gel F_{254}TLC plates (Merck) developed with $CHCl_3/CH_3OH$ 5:1 and observed under UV light (λ = 254 and 366nm). UV/VIS spectra were recorded with a Specord UV/VIS spectrophotometer in DMF. IR spectra were recorded with a FT-IR Bruker JFS-113 Spectrometer in KBr pallets. ^1H NMR and ^{13}C NMR spectra were determined with a Varian Gemini 300 (300 MHz) Spectrometer in DMSO-d_6 and deuterated TFA solutions with TMS as internal standard. Chemical shifts are given in the δ scale (ppm) and coupling constants in Hz. ^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. ^{13}C NMR (75.460) spectra were recorded with spectral width 18.76 kHz, acquisition time 1.0 s, recycle delay 1.0 s and pulse width 15 μs. Homonuclear ^1H-^1H shift correlated two-dimensional diagrams were obtained on Varian Gemini 300 spectrometer using the COSY pulse sequence. The spectral width was 4.97 kHz, acquisition time 0.206 s, number of increments in t_1 512 and number of scans 16. Heteronuclear 2D ^{13}C -^1H chemical shift correlation experiments were carried out using HETCOR spectra. The spectra were acquired with 2K data points, 245 increments and spectral width 19 kHz for ^{13}C and 4.97 kHz for ^1H.

Fluorescence corrected spectra were taken in DMF with a Perkin Elmer MPF-3 instrument. Fluorescence quantum yields were calculated by the equation $\Phi_f = (FA_s n^2 \Phi_s)/(AF_s n_0^2)$, where the subscripts refers to the standard and Φ is the quantum yield, F the corrected, integral fluorescence; A the absorption at the excitation wavelength, n the refractive index of DMF and n_0 the refractive index of H_2O. Quantum yield standards were quinine sulfate, 10 μM in 1 *N* H_2SO_4 (Φ=0.55) and 10 μM in 0.1 *N* NaOH (Φ=0.90) [25,26].

General Procedure for the Preparation of (*E*)-4-Chlorocarbonylalkoxystilbenes **1-8**.

A mixture of 0.015 mole of appropriate (*E*)-4-stilbenyloxyalkylcarboxylic acids and thionyl chloride (6 mL, 0.075 mole) in dry benzene (20 mL) was heated under reflux for 1 hr. Then the thionyl chloride and the solvent were evaporated *in vacuo*. The solid isolated was suspended in dry benzene and filtered trough silica gel (Merck 60 0.063-0.100mm). The solvent was removed again. Compounds **1**-**8** were shown to be analytically pure and were used without any further purification.

General Procedure for the Preparation of N^4-(E)-stilbenyloxyalkylcarbonyl-cytosines **9-16**.

Cytosine (1.11 g, 0.01 mole) and DMAP (*N*,*N*-dimethylaminopyridine 0.040 g 0.0003 mole) was suspended in dry pyridine (20 mL) and boiled under reflux. Then the boiling mixture was treated dropwise with the solution of 0.015 mole of appropriate (*E*)-4-chlorocarbonylalkoxystilbene **1**-**8** in dry benzene (2 mL). The reaction mixture was then allowed to reflux for 6 hrs. The solvent was then removed *in vacuo* and the solid was treated with dry methanol and boiled. The precipitated solid was collected by filtration from hot solvent. This procedure was repeated three times. Solids **9**-**16** were collected and dried under vacuum at room temperature. The physicochemical properties of **9**-**16** are given in Table I.

General Procedure for the Preparation of N^4-(*E*)-Stilbenyloxyalkylcarbonyl-1-methyl Cytosines **17-24**.

1-Methylcytosine (1.25 g 0.01 mole) and DMAP (*N*,*N*-dimethylaminopyridine 0.040 g, 0.0003 mole) was suspended in dry pyridine (20 mL) and boiled under reflux. Then the boiling mixture was treated dropwise with the solution of 0.015 mole of appropriate (*E*)-4-chlorocarbonylalkoxystilbene **1**-**8** in dry benzene (2 mL). The reaction mixture was then reflux for 5 hrs. Then the solvent was removed under vacuum, and the solid precipitated was treated with dry methanol and boiled. The solid of **17**, **19**, and **21** was collected by filtration from hot solvent and purified like described above. The compounds **18**, **20**, **22**, **23**, **24** were separated by column chromatography (silica gel Merck 60 0,063-0,100mm, $CHCl_3$:CH_3OH 30:1). The physicochemical properties of **17-24** are given in Table I.

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