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SILYL MODIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS

5.* HYDROLYTIC STABILITY AND BIOLOGICAL ACTIVITY OF THE TRIALKYLSILYL DERIVATIVES OF SOME HETEROCYCLIC BASES

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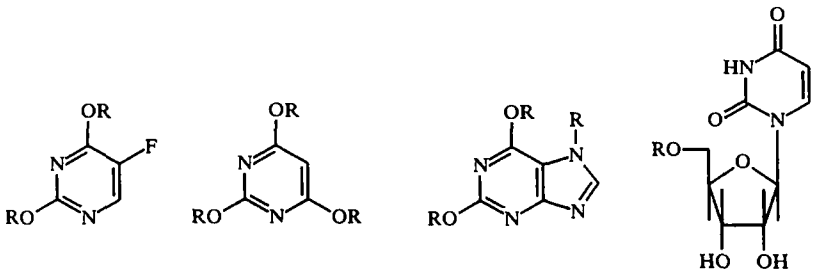
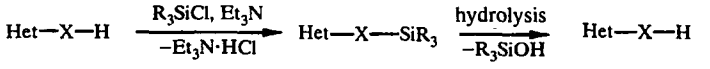
The kinetics of the desilylation of the triorganosilyl derivatives of some biologically active heterocyclic bases and uridine were investigated by ^1H NMR spectroscopy. A correlation was established between the relative rates of desilylation and the steric environment of the silicon atom. In trials on locomotor activity and muscular tone, the effect on memory processes, and the Porsolt test it was found that tris(tert-butylmethylsilyl)barbituric acid has higher sedative activity than barbituric acid. In contrast to uridine, 5'-O-tert-butyldimethylsilyluridine exhibits antitumor activity, suppressing the development of fibrosarcoma in human lungs (HT-1080) and fibroblasts in mice.

As a result of the hydrolytic lability of the Si–N and Si–O bonds [2] the triorganosilyl derivatives of biologically active compounds are most suitable for use as medicinal products [3]. The side products of hydrolysis, i.e., triorganosilanols and disiloxanes, are as a rule not very toxic substances [4]. The possibility of controlling the rate of desilylation by varying the substituents at the silicon atom is a characteristic feature of the compounds.



R = Me_3Si, Et_3Si, $(i\text{-}Pr)_3Si$, $t\text{-}BuMe_2Si$, Me_2TxSi (Tx = Me_2CHMe_2C)



Het—X—H 5-fluorouracil, barbituric acid, xanthine
X = O, N

*For communication 4, see [1].

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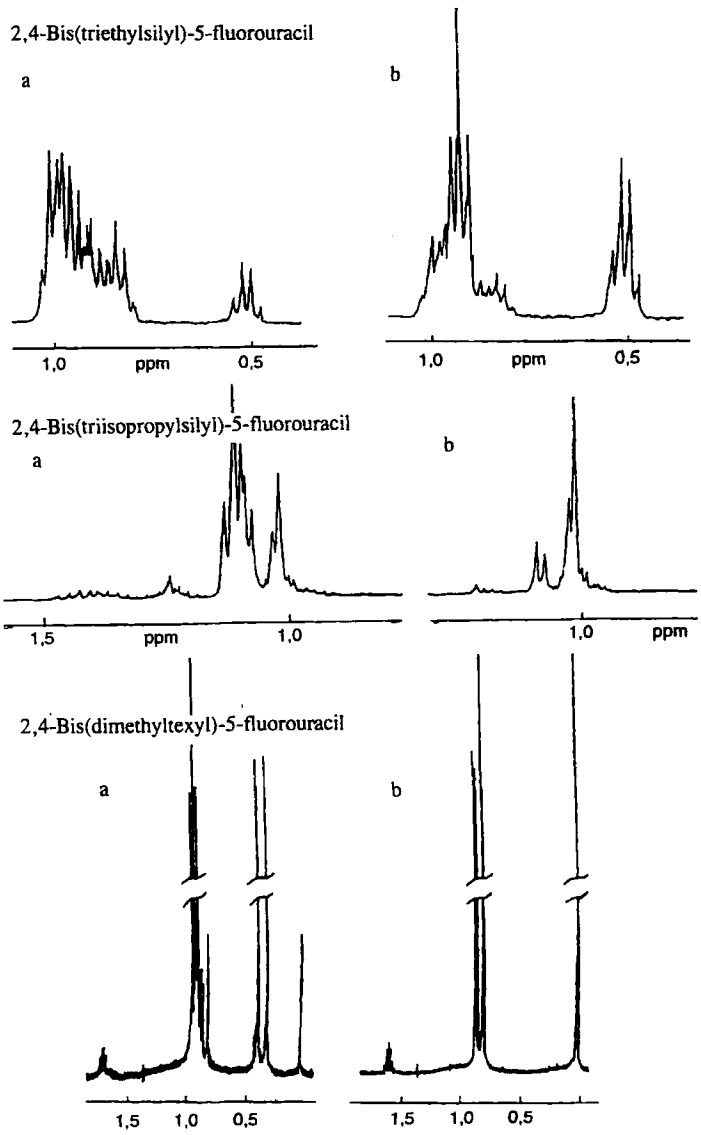


Fig. 1. ^{1}H NMR spectra (δ 0-1.5 ppm) of the trialkylsilyl derivatives of 5-fluorouracil: a) time t_i; b) time t_{ii}, $t_{ii} > t_i$.

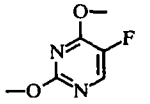
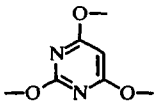
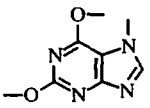
We synthesized the N- and O-triorganosilylated derivatives of biologically active heterocyclic bases (5-fluorouracil, barbituric acid, xanthine) and uridine and investigated their hydrolytic desilylation (scheme) with ^{1}H NMR control of the triorganosilyl spectral region.

Triorganosilylation was carried out with the triorganochlorosilanes in boiling benzene in the presence of triethylamine [5]. 5′-O-*tert*-Butyldimethylsilyluridine was obtained by the silylation of uridine with *tert*-butyldimethylchlorosilane in tetrahydrofuran in the presence of 1,4-diazabicyclo[2.2.2]octane and silver nitrate [6].

Hydrolytic desilylation was conducted in the dioxane–water system. The gradual decrease in the intensity of the signals in the silyl region of the ^{1}H NMR spectrum of the investigated compound was accompanied by an increase in the intensity of the signal of the hydrolysis product (trialkylsilanol) in the same region (Fig. 1). The hydrolysis of the silylated heterocyclic amines was characterized by the dependence of $\ln(C_0/C_t)$ on t (C_0 is the initial relative concentration, and C_t is the relative concentration of the substrate at the time of measurement t) (Fig. 2). This makes it possible to calculate the pseudofirst reaction rate constant (k') and the half-conversion time ($\tau_{1/2}$) (Table 1).

We established that the relative rate of hydrolysis depended on the steric environment of the silicon atom, which affects the rate of its attack by the hydroxide ion. The hydrolysis rate decreased with the silyl substituents in the following order: $Me_3Si- > Et_3Si- > i\text{-}Pr_3Si- > TxMe_2Si-$, which agrees with published data [7, 8] on the decrease in the rate of hydro-

TABLE 1. Results of the Kinetic Investigation of Hydrolysis (T = 37 ± 1°C)*

Compound Het—X—SiR_3†		k, sec^{-1}	$\tau_{1/2}$, min
	R = Et	$5{,}09 \cdot 10^{-3}$	2,27
	R = *i*-Pr	$4{,}12 \cdot 10^{-3}$	2,81
	R_3 = TxMe2	$3{,}64 \cdot 10^{-3}$	3,18
	R = Et	$3{,}93 \cdot 10^{-3}$	2,94
	R = *i*-Pr	$2{,}62 \cdot 10^{-3}$	4,40
	R_3 = TxMe2	$9{,}76 \cdot 10^{-3}$	11,08
	R = Et	$4{,}26 \cdot 10^{-3}$	2,72
	R = *i*-Pr	$3{,}12 \cdot 10^{-3}$	3,70
	R_3 = TxMe2	$1{,}47 \cdot 10^{-3}$	7,83

*The calculations were performed with the following relationships:

$$a >> b,\quad k = \frac{1}{t_a} \cdot \ln\frac{b}{b-x},\quad k' = ka,\quad \tau_{1/2} = \frac{0.6931}{k'}$$

a and b are the initial concentrations of the reactants (b — [Het−X−SiR_3], a — [H_2O]); $a - x$ and $b - x$ are the current concentrations of the reactants; k is the reaction rate constant; $\tau_{1/2}$ is the half-conversion time.

†X = O, N.

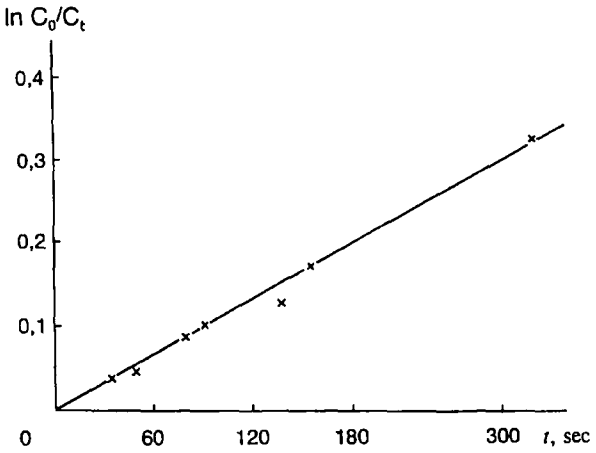


Fig. 2. Dependence of the ln C_0/C_t parameters on t for the hydrolysis of 2,4,6-tris(dimethyltexylsilyl)barbituric acid.

lysis of the trialkylsilyl derivatives of ephedrine, norephedrine, and methylephedrine with increase in the size of the organic radicals attached to the silicon atom.

The electronic nature of the heterocyclic systems themselves also has a significant effect on the hydrolysis rate. Thus, the latter decreases in the transition from the silyl derivatives of 5-fluorouracil to xanthine and then to barbituric acid.

Since the silylation of the compounds increases their lipophilicity, the triorganosilylated drugs could be used to treat pathological processes requiring the intracellular action of the products or their penetration across the hematoencephalic barrier [9]. The effectiveness of this process depends on the rate of hydrolysis of the silylated compound.

We investigated the effect of 2,4,6-tris(*tert*-butyldimethylsilyl)barbituric acid on the central nervous system and studied the antitumor activity of 5′-O-*tert*-butyldimethylsilyluridine. The *tert*-butyldimethylsilyl groups, which temporarily block the hydrophilic functions and do not undergo instant hydrolysis, were chosen on the principle of the commercial availability of *tert*-butyldimethylchlorosilane. The results of the biological trials were compared with data obtained for the unsilylated precursors.

TABLE 2. Toxicity and Psychotropic Activity of Barbituric Acid (B) and 2,4,6-Tris(*tert*-butyldimethylsilyl)barbituric Acid (B−Si)

Test	ED_{50}, mg/kg (or % in relation to control, 100%)	
	B	B—Si
LD_{50}	2740	3250
Tube	274	258
Pull up on cross bar	>500	355
Hypoxic hypoxia	112	118
Phenamine hyperactivity	78/89	78*/100
Corazole seizures	108/164*	126*/149*
Conditional reflex of passive avoidance	103,8	140,8*
Retrograde amnesia	83,3	100,0
Porsolt	76*	80*

*The differences in relation to the control are statistically reliable at $P \leq 0.05$.

TABLE 3. Antitumor Activity* of 5′-O-*tert*-Butyldimethylsilyluridine on Cultures of Cells HT-1080 (fibrosarcoma in human lungs) and NiH 3T3 (fibroblasts in mice)†

Concentration, μg/ml	Perished cells, %			
	HT-1080		NiH 3T3	
	CV	MTT	NR	MTT
100	84	96	88	95
25	26	18	41	41
6,3	48	-5	10	28
1,6	63	-3	10	18
0,4	69	-2	-19	10

*CV = crystal violet; NR = neutral red; MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide.
†Uridine does not exhibit cytotoxicity in these tests.

As a result of the investigation it was found that the silylated and unsilylated barbituric acids exhibit sedative activity (Table 2), which manifests itself in the tests on the locomotor activity and muscular tone and is somewhat more clearly defined when 2,4,6-tris(*tert*-butyldimethylsilyl)barbituric acid is used. In the "pull up on a cross bar" test the silylated barbituric acid is 1.5 times more active than the unsilylated compound. Barbituric acid and its silylated product are antagonists of corazole and raise the threshold of corazole seizures. However, barbituric acid exhibited high activity in the tonic phase (108/164), while the silylated compound exhibited high activity in the clonic phase (126/149).

2,4,6-Tris(*tert*-butyldimethylsilyl)barbituric acid had the strongest effect on the memory processes, fully suppressing (100%) retrograde amnesia and doubling the latent learning period; the indices for barbituric acid are lower (by 83.3% and 1.4 times respectively). Silylated barbituric acid exhibits somewhat higher antistress activity than the unsilylated compound in the Porsolt test. Both compounds have low toxicity; the acute toxicity index of silylated barbituric acid is 1.2 times lower.

5′-O-*tert*-Butyldimethylsilyluridine, unlike uridine, exhibits antitumor activity (Table 3), suppressing the development of fibrosarcoma in human lungs (HT-1080) and mice fibroblasts (NiH 3T3) and also tumor cells by 84-96% at a concentration of 100 μg/ml and by 70% (HT-1080, CV-test) at a concentration of 0.4 μg/ml.

Thus, the comparative study of silylated barbituric acids and uridine and their unsilylated precursors indicates that the indices of psychotropic activity of 2,4,6-tris(*tert*-butyldimethylsilyl)barbituric acid nevertheless exceed those obtained for barbituric acid in most of the tests carried out. 5′-O-*tert*-butyldimethylsilyluridine is active in the tests on antitumor activity with the complete absence of activity for uridine.

As a result of the silyl modification of drugs [3] the type of biological activity can either remain unchanged [10], as in the case of barbituric acid, or change [11], as in the case of uridine. If the type of biological activity remains, its level can

change as a result, probably, of the relative stability of the Si–O and Si–N bonds. The insignificant difference between 2,4,6-tris(*tert*-butyldimethylsilyl)barbituric acid and the unsilylated precursor in the tests on psychotropic activity indicates that the selected trialkylsilyl group in this case is not optimal. Nevertheless, for the silyl-modified compounds that exhibit biological activity even at the level of their unsilylated precursors and have as a rule lower toxicity the therapeutic index is higher while the effective dose, calculated on the active substance by weight, is less than for the unsilylated compound. This represents a distinct advantage over the expensive more toxic products that exhibit habituation.

The obtained data make it possible to conclude that O- and N-trialkylsilyl groups can be used successfully in the design of drugs.

EXPERIMENTAL

The PMR spectra were recorded on a Bruker WH-90/DS instrument in dioxane-d_8 with TMS as internal standard.

General Procedure for the Determination of the Hydrolysis Rate of Silylated Heterocyclic Bases. The silylated heterocyclic bases (15-20 mmole) were placed in the NMR sample tube and dissolved in dioxane (0.6 ml). After the addition of 0.4 ml of water and rapid agitation the changes in the spectral region of δ 1.5-0.0 ppm were recorded at specific time intervals. The initial concentration of the initial compound C_0 was taken as the sum of the intensities of the signals for the initial compound and the hydrolysis product. The decreasing intensity of the signal of the initial compound was used as the concentration of the compound C_t at time t. The pseudofirst hydrolysis rate constant (k') and the half-conversion time ($\tau_{1/2}$) were calculated from the dependence of ln (C_0/C_t) on t. The hydrolysis rate was determined at constant temperature (37 ± 1°C) and pressure.

The cytotoxic activity of the compounds was estimated by the usual procedures [12-14] from the effective concentration (μg/ml) suppressing the growth of the cells by 50%. The neurotropic activity of the compounds was assessed on mice of the BALB/c line and on mongrel male rats. An oil solution of the investigated substance was introduced intraperitoneally 30 min before the trial [15]. The experimental data were processed statistically by the express method [16]. The acute toxicity of the compounds was determined in trials on mongrel male rats with intraperitoneal injection.

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