New acid photogenerators based on thioxanthen-9-one sulfonium derivatives for detritylation in the oligonucleotide synthesis

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Photochemical activity of a number of cationic thioxanthen-9-one derivatives for the formation of the trityl cation was studied, which resulted in the selection of compounds suitable for photodetritylation. 10-(4-Heptyloxyphenyl)-9-oxo-2-(*N*,*N*,*N*-triethylammonio)methyl-9*H*-thioxanthenium bis(hexafluorophosphate) and 2-methyl-, 2-(2-methyl-1-propanoyl-2-tosyl)-, 1-chloro-4-propoxy-, and 2,4-diethyl-10-(4-heptyloxyphenyl)-9-oxo-9*H*-thioxanthenium hexafluorophosphates were found to be photoactivators of detritylation of 5´-*O*-(4,4´-dimethoxytrityl)thymidine. The detritylation reaction is the most efficient in dichloromethane. 2,4-Diethyl-10-(4-heptyloxyphenyl)-9-oxo-9*H*-thioxanthenium hexafluorophosphate was used as a detritylation photoactivator in the oligonucleotide synthesis using an automated DNA synthesizer. The yield in the elongation step of the oligonucleotide chain was 98%.

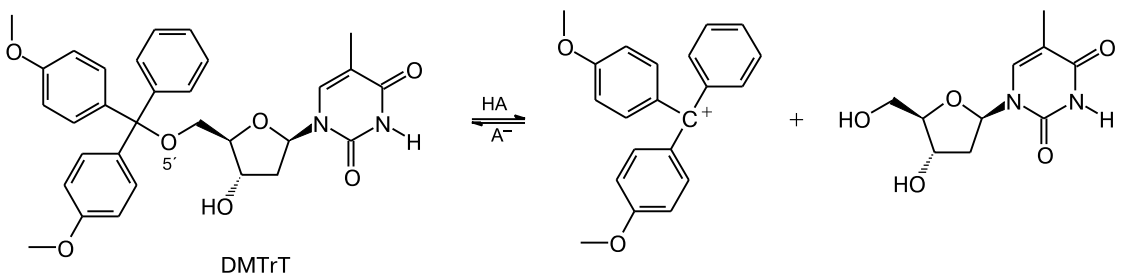
Key words: photoactivators of detritylation, acid photogenerators, trityl cation, thioxanthenone derivatives.

The present-time formation and development of synthetic biology is based on the breakthrough in the field of oligonucleotide synthesis. The target genetic constructions obtained for the synthesis of various genomes exclusively consist of synthetic oligonucleotides.[1—4] The DNA microchips are now used for the synthesis of oligonucleotides required for the construction of genetic elements.[5] One of the promising approaches to the removal of protecting groups in the oligonucleotide synthesis on the chip surface consists of the use of UV irradiation. In particular, photogenerated acids are used for the removal of 5´-dimethoxytrityl protecting group in the oligonucleotide synthesis.

Abstraction of the dimethoxytrityl cation from the nucleoside with dimethoxytrityl protecting group, in our case it is 5´-*O*-(4,4´-dimethoxytrityl)thymidine (DMTrT), is an acid-catalyzed process, which proceeds according to Scheme 1.[6] The nucleoside residue with the free 5´-OH group formed is further involved in the oligonucleotide chain elongation according to the known amidophosphite approach to the synthesis of polynucleotides.[7,8]

On detritylation, removal of dimethoxytrityl protecting groups is effected by an acid formed in the course of a photochemical reaction. A search for the acid photogenerators (APG) efficient in the detritylation reaction is

Scheme 1



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a topical problem. Earlier,[9,10] triarylsulfonium and diaryliodonium hexafluoroantimonates, as well as halo-substituted triazines have been suggested as detritylating acid photogenerators. Targeted synthesis of a number of 2-nitrobenzyl trichloroacetates was performed[11] to be employed for the photoinduced detritylation. Their photolysis in dichloromethane occurs with the quantum yields of photodecomposition from 0.01 to 0.8 depending on the structure.[12]

Synthesis of a number of thioxanthenium salts, which are used as versatile photoinitiators, is also described.[13,14] However, their properties as photoactive compounds for the acid photogeneration and detritylation of trityl-substituted nucleotides have not been studied.

In the present work, we studied the possibility of using thioxanthen-9-one sulfonium derivatives as acid photogenerators and selected compounds suitable for the formation of trityl cation from the trityl-substituted nucleoside DMTrT.

Experimental

The system of photochemical detritylation included a solution of a photoinitiator, cationic thioxanthen-9-one derivative (CTD), and a nucleoside with the dimethoxytrityl protecting group, *viz.*, 5′-*O*-(4,4′-dimethoxytrityl)thymidine, in chloroform, dichloromethane, or acetone. 5′-*O*-(4,4′-Dimethoxytrityl)thymidine (PA Vostok, Russia) was additionally purified by column chromatography on silica gel in the gradient $CHCl_3$—EtOH (0—10%). The CTD hexafluorophosphates **1**—**8** (see Refs. 13 and 14) differing in the structure of the cationic moieties of the molecules were studied as photoinitiators: compounds **1**—**4** are sulfonium salts, **5** is an ammonium salt, **6** is a phosphonium salt, **7** is a sulfonio-ammonium salt, and **8** is a sulfonio-phosphonium salt.

A DKsSh-1000 xenon lamp was used for the UV irradiation of solutions, the irradiation with $\lambda = 375$ nm was selected using an MDR-2 diffraction monochromator. Absorption spectra were recorded on a Hewlett—Packard 8453 spectrophotometer, spectra of solutions during irradiation were recorded on an Avantes AvaSpec-3648 spectrophotometer. Kinetic curves of the change in the optical density of solutions in the absorption maxima of the starting compounds and their photodecomposition products and in the absorption maximum of the trityl cation ($\lambda = 506$ nm) were plotted based on the data from the changes in absorption spectra of the solutions.

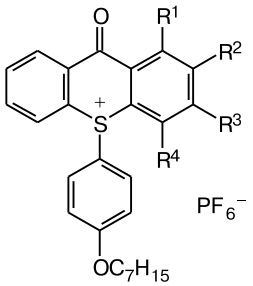
GLC-MS analysis of the APG photolysis products was performed on a Hewlett—Packard instrument including an HP 5890 Series II gas chromatograph and an HP 5971 mass-selective detector (EI, 70 eV); the HP-5 capillary column, poly(5%-phenyl—methylsiloxane) as a stationary liquid phase, and helium as a carrier gas were used.

An ASM-800 DNA synthesizer (BIOSSET Ltd, Russia) was used for the synthesis of oligonucleotides. The synthesizer was modified for performing partially automated protocol in the synthesis of oligodeoxyribonucleotides on a 0.5 micromolar scale on a porous glass in a flowing-through reator with a photochemical step. For the removal of 5′-*O*-(4,4′-dimethoxytrityl) group, a 1% solution of 2,4-diethyl-10-(4-heptyloxyphenyl)-9-oxo-9*H*-thioxanthenium hexafluorophosphate was placed into the synthesizer vessel instead of trichloroacetic acid.[4]

Oligonucleotide synthesis was carried out in quartz columns (10×2.5 mm, internal diameter 1 mm) containing porous glass with the covalently bound first nucleotide unit. The synthesizer was equipped with a high pressure mercury lamp (DRK-120) and an OI-18A lightening optical block (LOMO, Russia) containing a shatter, a filter holder, and lenses for the selection and focusing of the mercury lamp emission. The BS-7 and UFS-1 light filters ($\lambda = 365$—390 nm, $W = 2$ mW cm^{-2}) were used. Detritylation was repeated three times, the exposition time for the photoformation of each portion of the acid was 1 min. The subsequent steps of the oligonucleotide chain elongation using the amidophosphite approach including capping of remaining 5′-hydroxy groups with acetic anhydride were carried out according to the standard protocol of automated oligonucleotide synthesis. After the synthesis was completed, the support was treated with concentrated aqueous ammonia at 60 °C for 4 h. The reaction mixtures of the synthesized oligonucleotides were analyzed by HPLC and gel-electrophoresis in polyacrylamide gel (PAAG).

HPLC was performed on a Milikhrom A-02 chromatograph, using a ProntoSil-120-5-C18 AQ column with the particle sizes 5 μm, a linear gradient of acetonitrile (2—20%) in 0.05 *M* triethylammonium acetate buffer (pH 7.5) was used over 10 min with the elution rate of 150 μL min^{-1}.

The mass spectrum of dAAAAAAAAAA was recorded on an Autoflex II mass spectrometer (Bruker Daltonics, Germany) with



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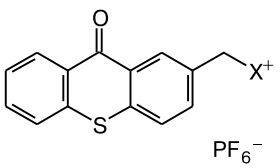

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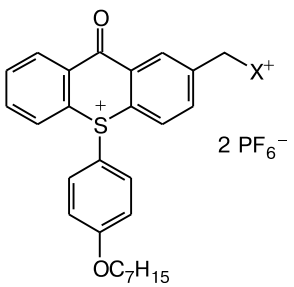

7, 8

$R^1 = R^3 = R^4 = H$, $R^2 = C(O)CMe_2Ts$ (**1**); $R^1 = Cl$, $R^2 = R^3 = H$, $R^4 = OPr$ (**2**); $R^1 = R^3 = R^4 = H$, $R^2 = Me$ (**3**); $R^1 = R^3 = H$, $R^2 = R^4 = Et$ (**4**); X = NMe_2CH_2Ph (**5**), PPh_3 (**6**), NEt_3 (**7**), PPh_3 (**8**)

the flexControl 2.4 program package. The calibration of the mass spectrometer in the negative linear mode was performed using a set of oligonucleotides (Part No. 20 62 00, Bruker Daltonics, Germany). A solution of 3-hydroxypicolinic acid (50 mg mL^{-1} in water—acetonitrile (1 : 1)) with addition of ammonium citrate to the concentration 10 mg mL^{-1} was used as a matrix. MS of dAAAAAAAAAA, m/z, found: 3070.400; calculated: 3070.135.

Results and Discussion

Spectroscopic characteristics of cationic thioxanthenone derivatives and their photodecomposition products. The absorption spectra of CTD photodecomposition products were obtained and their extinction coefficients were calculated in the UV irradiation of solutions of the starting compounds ($4.1 \cdot 10^{-4}$ mol L^{-1}) in chloroform or dichloromethane until complete photodecomposition was reached. Typical absorption spectra of compound **4** and its photodecomposition products are given in Fig. 1.

All the CTD studied have an average extinction coefficient from 500 to 1000 L mol^{-1} cm^{-1} depending on the structure of the compound in the region 350—405 nm generated by the compact UV lasers. The photodecomposition products possess stronger absorption bands in this region of the spectrum with the extinction coefficient from 1000 to 5000 L mol^{-1} cm^{-1}. According to the GLC-MS data for CTD **4** photolyzate, heptyl phenyl ether (m/z = 192) and 2,4-diethylthioxanthen-9-one (m/z = 268) are the major photodecomposition products. An increase in absorption of the photolysis product of compound **4** (see Fig. 1) in the region 350—400 nm indicates the accumulation of 2,4-diethylthioxanthen-9-one. The absorption spectrum of photolyzate of compound **1** in the region 350—400 nm corresponds to the absorption spectrum of 2-methylthioxanthen-9-one.

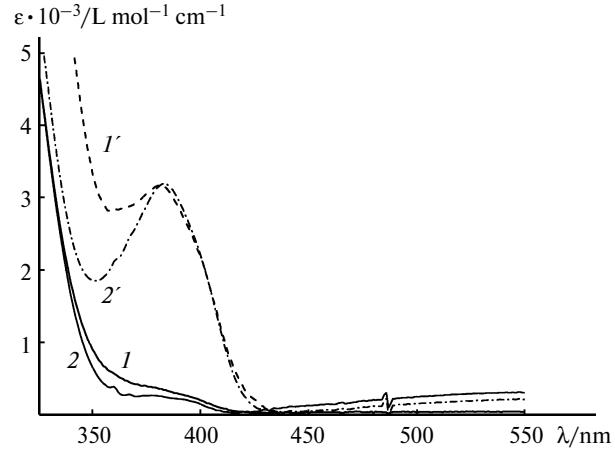

Fig. 1. Absorption spectra of APG **4** (*1*, *2*) and its photodecomposition products (*1′*, *2′*) in chloroform (*1*, *1′*) and dichloromethane (*2*, *2′*).

According to the mechanism of photodecomposition of sulfonium salts,[15,16] the intermediates of both the radical and the cationic type form as the reaction products, the acid of the corresponding anion being the major final photolysis product (Scheme 2). Thus it was shown[16] that photolysis of triarylsulfonium chloride leads to hydrogen chloride (57%), diaryl sulfide (26%), benzene (10%), and ethoxybenzene (9%); other products were present in trace amounts.

Scheme 2

$$Ar_3S^+X^- \overset{h\nu}{\rightleftharpoons} (Ar_2S^{\cdot+}, Ar^{\cdot})X^- \longrightarrow Ar_2S^{\cdot+} + Ar^{\cdot} + X^-$$

$$Ar_2S^{\cdot+} + R{-}H \longrightarrow Ar_2S^+{-}H + R^{\cdot}$$

$$Ar_2S^+{-}H \longrightarrow Ar_2S + H^+$$

Taking into account the general scheme of photodecomposition of triphenylsulfonium salts[15] to the sulfide and phenyl fragments and considering the data from absorption spectra and GLC-MS for the photolysis products of compound **4**, we assume that hexafluorophosphoric acid, the corresponding thioxantenones, and heptyl phenyl ether are the major photodecomposition products of compounds under study.

No compounds that absorb in the visible region of the spectrum are found among the photolysis products, which could interfere with the spectrophotometric detection of the trityl cation ($DMTr^+$) possessing absorption in the green region of the spectrum[17] with λ_{max} = 506 nm and the extinction coefficient $\varepsilon = 7 \cdot 10^4$ L mol^{-1} cm^{-1}.

Kinetics of photochemical detritylation. The formation of the trityl cation from DMTrT under the action of an acid photogenerator occurs in two steps.[18] In the first step, the acid photogenerator decomposes to yield hexafluorophosphoric acid and other reaction products. In the second step, DMTrT reacts with hexafluorophosphoric acid resulting in the trityl cation and thymidine (Scheme 3).

Scheme 3

$$APG \xrightarrow{h\nu} HPF_6 + P$$

$$DMTrT + HPF_6 \underset{k_3}{\overset{k_2}{\rightleftharpoons}} DMTr^+ + P$$

APG, acid photogenerator; P, product

Kinetics of the photochemical transformation of CTD and DMTrT in chloroform, dichloromethane, or acetone was studied by subjecting the solutions to the UV irradiation at λ = 375 nm in quartz cells (1×1×1 cm). The spec-

tral changes upon photo-induced accumulation of the trityl cation in the system CTD **4** ($C = 1.25 \cdot 10^{-3}$ mol L^{-1})—DMTrT ($C = 3.6 \cdot 10^{-5}$ mol L^{-1}) in dichloromethane are shown in Fig. 2. An increase in absorption at 506 nm corresponds to the increase in the concentration of the trityl cation: the starting compound and its photodecomposition products absorb in the UV region.

To determine the operating concentration of CTD, we studied the dependence of photodetritylation kinetics on the ratio of concentrations of CTD **3** and DMTrT. The concentration of CTD **3** in chloroform was varied from $3.6 \cdot 10^{-5}$ mol L^{-1}, which is equal to the concentration of DMTrT for spectrophotometric studies (as determined earlier[18]), to 10^{-2} mol L^{-1}. Figure 3, *a* shows the kinetic curves of accumulation of the dimethoxytrityl cation in a solution upon irradiation by the incident light with the intensity 800 μW cm^{-2} averaged over four independent measurements. Based on the data obtained, the dependence of the maximum reaction rate of detritylation on the concentration of CTD (Fig. 3, *b*) was plotted, from which it follows that at the concentration above $3 \cdot 10^{-3}$ mol L^{-1} the curve of the reaction rate reaches saturation. This is due to the fact that optical density of the solution for this concentration exceeds 1.5, and further increase in the concentration of CTD does not lead to the increase in the amount of the absorbed light. The dependence of the accumulation rate of $DMTr^+$ (V_{DMTr}) on the concentration of CTD (C) for a given intensity of light is generally described by the expression

$$V_{DMTr} = \varphi I(1 - 10^{C\varepsilon d}),$$

where ε is the molar extinction coefficient of CTD on the wavelength of irradiation; d is the optical path, φ is the quantum yield, I is the intensity of the absorbed light.

The efficiency of photodetritylation was determined for the concentration of CTD $1.25 \cdot 10^{-3}$ mol L^{-1}, which is close to the limit of solubility of some compounds and to the concentration of DMTrT ($3.6 \cdot 10^{-5}$ mol L^{-1}).

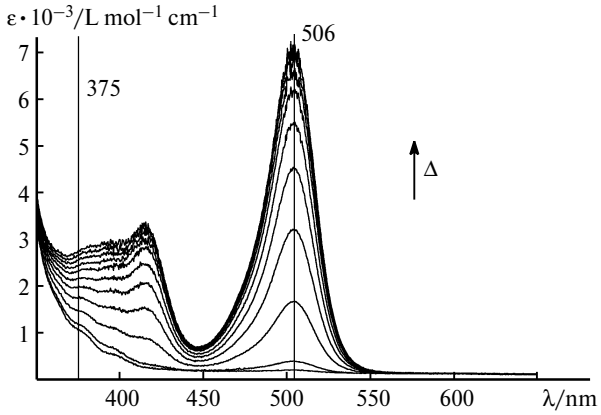

Fig. 2. Spectral changes in the photochemical accumulation of the trityl cation in the system APG **4** ($C = 1.25 \cdot 10^{-3}$ mol L^{-1})—DMTrT ($C = 3.6 \cdot 10^{-5}$ mol L^{-1}); Δ is the time interval of 5 s.

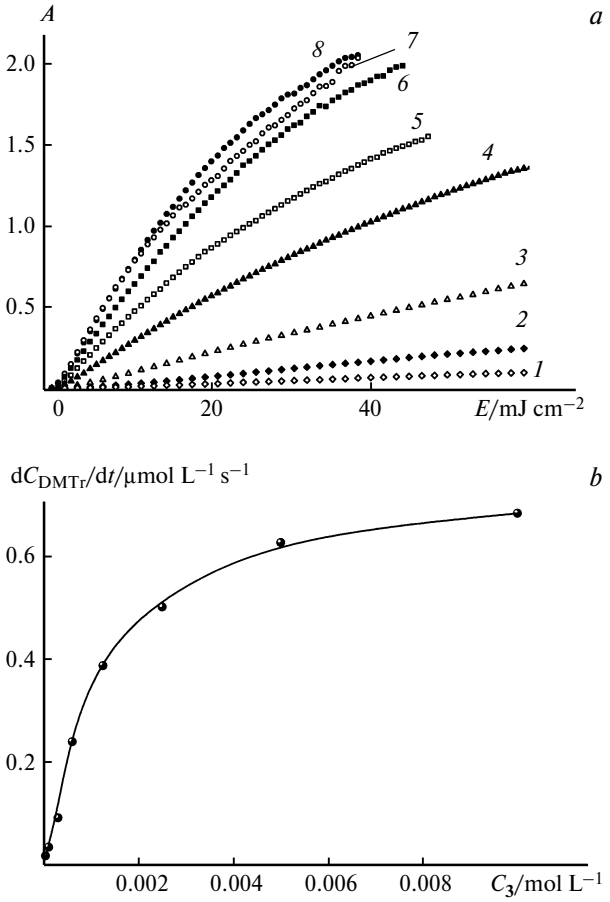

Fig. 3. (*a*) Kinetic curves of the optical density change of solutions at λ = 506 nm during accumulation of $DMTr^+$ upon photolysis of APG **3** at λ = 375 nm for the concentration of APG **3** in dichloromethane $0.036 \cdot 10^{-3}$ (*1*), $0.1 \cdot 10^{-3}$ (*2*), $0.3 \cdot 10^{-3}$ (*3*), $0.6 \cdot 10^{-3}$ (*4*), $1.25 \cdot 10^{-3}$ (*5*), $2.5 \cdot 10^{-3}$ (*6*), 5μ $\cdot 10^{-3}$ (*7*), and $1 \cdot 10^{-4}$ mol L^{-1} (*8*). (*b*) The maximum rate of detritylation reaction versus concentration of APG **3**.

Kinetics of the solvent dependence of $DMTr^+$ accumulation was studied by performing photolysis of CTD **3** in the presence of DMTrT in different solvents: chloroform, dichloromethane, and acetone. The curves of the changes of optical density in the $DMTr^+$ absorption maximum are given in Fig. 4.

As is seen from the Fig. 4, the character of $DMTr^+$ accumulation significantly depends on the solvent. Photogeneration of $DMTr^+$ in acetone is very weak. Optical density at λ = 506 nm does not virtually rise. In chloroform, optical density of $DMTr^+$ rises rapidly up to the exposition 150 mJ cm^{-2}, reaches the maximum, and slowly decreases on further irradiation. Presumably, further photolysis of the CTD photoproducts in chloroform leads to the destruction of $DMTr^+$. Unlike chloroform, photoaccumulation of $DMTr^+$ in dichloromethane occurs rather efficiently and without subsequent decomposition of $DMTr^+$ formed. Therefore, dichlo-

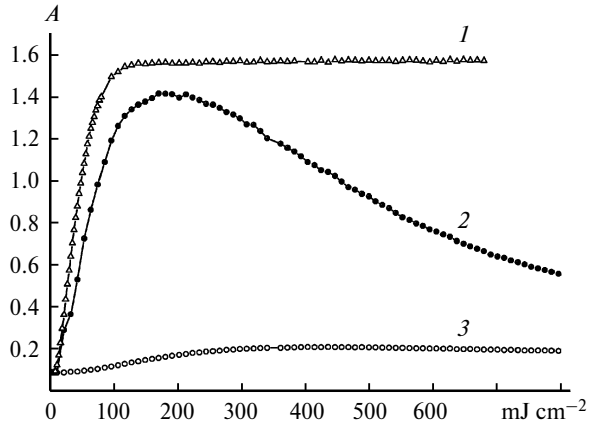

Fig. 4. Curves of the optical density change upon the UV irradiation at λ = 506 nm of solutions of APG **3** in the presence of DMTrT in dichloromethane (*1*), chloroform (*2*), and acetone (*3*).

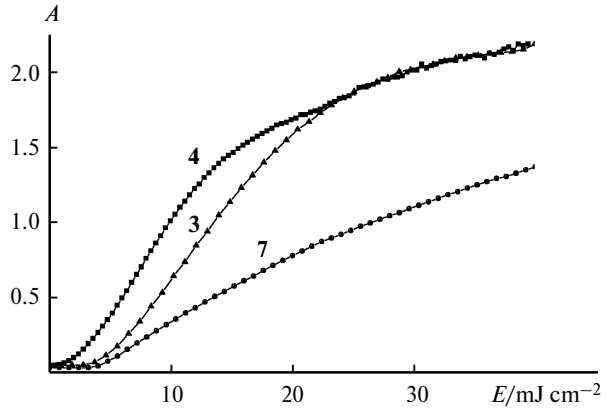

Fig. 6. Curves of the optical density change of solutions of DMTrT—APG **3**, **4**, and **7** in dichloromethane at λ = 506 nm upon the UV irradiation with λ = 370 nm.

romethane is a solvent of choice for photodetritylation of DMTrT.

The averaged kinetic curves of photoaccumulation of $DMTr^+$ in chloroform are given in Fig. 5. Since compound **7** is insoluble in chloroform, the curves of photoaccumulation of $DMTr^+$ for compounds **3**, **4**, and **7** were studied in dichloromethane (Fig. 6). As is seen from the given plots, significant increases in optical density in the absorption maximum of the trityl cation (λ = 506 nm, $A > 1.2$) as a result of photochemically inducted reaction of DMTrT with hexafluorophosphoric acid were recorded for the solutions containing CTD **1**, **2**, **3**, and **4**. These compounds are efficient APG. Solutions with compounds **5**, **6**, and **8** did not show any changes in optical density in the region of absorption of the trityl cation, and these compound can be considered as inefficient APG. The rates of photoaccumulation of $DMTr^+$ for APG **1**, **2**, **3**, and **4** are close, however, APG **4** is the most active. Solutions of CTD **3**, **4**, and **7** in dichloromethane showed high rates of accumulation of $DMTr^+$ and high values of its final optical density.

For APG **3** and **4**, proved the most efficient photoactive detritylating compounds, kinetic curves of APG consumption and photolysis product accumulation were recorded upon the UV irradiation with λ = 375 nm in chloroform and dichloromethane in the absence of DMTrT (Fig. 7).

The kinetic data on the change in optical density of solutions (see Figs 5—7) were used to calculate the quantum yields of the formation of photolysis products of APG (φ_P) and the trityl cation (φ_{DMTr}) for different APG in the initial step of product accumulations.[19] The quantum yields were calculated with allowance for the overlap of the absorption spectra of APG and their photolysis products.

The average values of quantum yields of the formation of photolysis products of APG and the trityl cation calculated from the initial linear sections of the kinetic curves

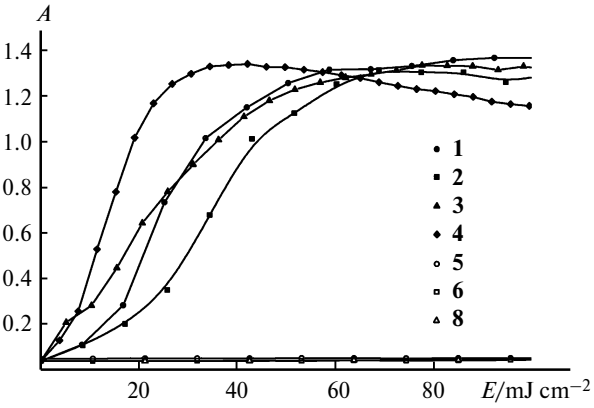

Fig. 5. Curves of the optical density change of solutions of DMTrT—APG **1**—**6** and **8** in chloroform at λ = 506 nm upon the UV irradiation.

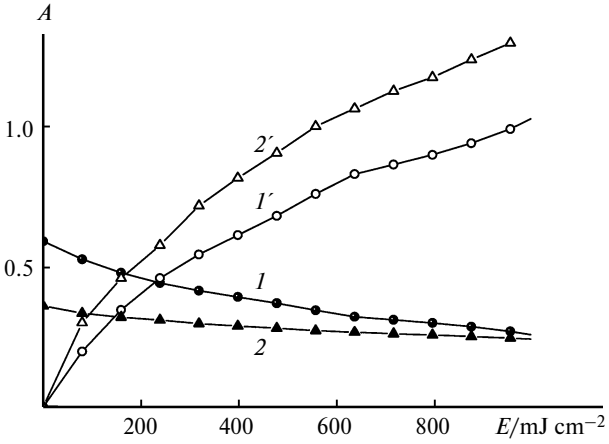

Fig. 7. Curves of the optical density changes of APG **3** (*1*) and **4** (*2*) at λ = 375 nm during photolysis and photolysis products of APG **3** (*1′*) and **4** (*2′*) at λ = 390 nm during photoaccumulation.

Table 1. Quantum yields of the formation reactions of hexafluorophosphoric acid and the trityl cation in chloroform (I) and dichloromethane (II) for CTD*

CTD	ε/L mol^{-1} cm^{-1}				φ_P		φ_{DMTr}	
	APG at λ = 375 nm		Product at λ = 375 nm					
	I	II	I	II	I	II	I	II
1	540	—	3400	—	—	—	0.13	—
2	4730	4970	2790	2800	0.33	0.35	0.13	0.04
3	500	470	2750	1520	0.46	0.88	0.13	0.36
4	400	300	3160	2870	0.60	0.80	0.27	0.4
5	5130	—	4960	—	—	—	$2.0 \cdot 10^{-4}$	—
6	3900	—	3350	—	—	—	$2.8 \cdot 10^{-3}$	—
7	—	600	—	3860	—	0.81	—	0.59
8	350	—	2600	—	—	—	$6.9 \cdot 10^{-3}$	—

* The errors in determination of extinction coefficients and quantum yields were 50 L mol^{-1} cm^{-1} and 20%, respectively.

are given in Table 1. The quantum yields of phototransformation of sulfonium derivatives **3**, **4**, and **7** in dichloromethane are close to unity. The quantum yield of accumulation of $DMTr^+$, as a rule, is lower than in the case of phototransformation of APG and depends on the APG structure. Apparently, this is due to either the specificity of acid elimination during photodecomposition of APG of different structures, or additional reactions of the photolysis products of APG with HPF_6, which are not shown in Scheme 3. It should be noted that the acid formed on photolysis of APG **7** is consumed almost completely ($\varphi_P = 0.81$, $\varphi_{DMTr} = 0.59$). The APG **4** showed relatively high quantum yield of the formation of the trityl cation, $\varphi_{DMTr} = 0.4$. Thioxanthen-9-one derivatives containing either only ammonium or phosphonium substituent, or sulfonio-phosphonium substituent, are inactive as APG for the detritylation reaction. In acetone, the quantum yields of detritylation are considerably lower. The quantum yield of the formation of the trityl cation for APG **3** in acetone calculated for comparison was $\varphi_{DMTr} = 0.005$ (*cf.* $\varphi_{DMTr} = 0.36$ in dichloromethane).

Taking into account the two-step scheme of accumulation of the trityl cation under the action of the acid photogenerator (see Scheme 3), an initial system of differential kinetic equations (1) was set for the key reactants (APG, HPF_6, DMTrT, and $DMTr^+$) for the change in the concentration of $DMTr^+$ in the initial period of detritylation, when the photochemical step is described by the equation of the zero order.[19,20]

The solution for the system of differential kinetic equations (1) by the Rosenbrock parametric method for the stiff set of ordinary differential equations allowed us to determine that the concentration of HPF_6 reaches the quasi-steady-state values ~$4 \cdot 10^{-6}$ mol L^{-1} after ~4 s and further changes insignificantly (Fig. 8). The literature data[18] were used as the initial values of the rate constants for the direct and reverse detritylation reactions. Such

$$\begin{cases} d[APG]/dt = -\varphi I \\ d[HPF_6]/dt = \varphi I - k_2[HPF_6][DMTrT] + \\ \qquad + k_3[DMTr^+][P] \\ d[DMTrT]/dt = -k_2[HPF_6][DMTrT] + \\ \qquad + k_3[DMTr^+][P] \\ d[DMTr^+]/dt = k_2[HPF_6][DMTrT] - \\ \qquad - k_3[DMTr^+][P] \\ d[P]/dt = k_2[HPF_6][DMTrT] - \\ \qquad - k_3[DMTr^+][P] \end{cases} \quad (1)$$

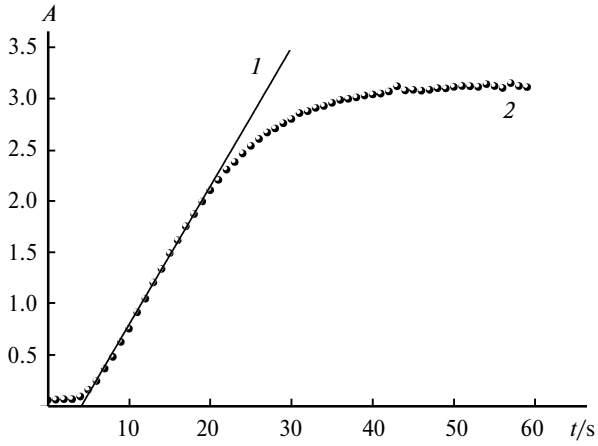

Fig. 8. Plot of the $y(t) = k_1(t - t_0)$ dependence (*1*) and experimental data on the change in the concentration of the trityl cation with time upon UV irradiation ($P = 2.65$ mW cm^{-2}, λ = 375 nm) of solutions of DMTrT and APG **4** in dichloromethane (*2*). The data were obtained for the optical density of the solution being registered at the wavelength 506 nm.

a kinetics of accumulation of hexafluorophosphoric acid allowed us to use the approximation of its quasi-steady-state concentration.[21] In this approximation, the system of differential equations (1) has a linear solution for the change in the concentration of the trityl cation:

$$d[DMTr^+]/dt = \varphi I(t - t_0). \quad (2)$$

The experimental curve of the change in the concentration of the trityl cation (*2*) upon the UV irradiation of solutions of DMTrT + APG **4** in dichloromethane is shown in Fig. 8. The straight line *1* fits the equation (2), where t_0 = 4.1 s is the time interval where the quasi-steady-state concentration is established, and φI = $= 1.4 \cdot 10^{-6}$ mol L^{-1} s^{-1}. As is seen from Fig. 8, the linear section of the the trityl cation concentration growth is well described in the approximation of quasistationary concentration of hexafluorophosphoric acid.

Cationic thioxanthenone derivatives as APG in the synthesis of oligonucleotides. The CTD **1—4** and **7** manifest high quantum yields of the formation of $DMTr^+$ from DMTrT and can be recommended as efficient APG for photodetritylation. Among CTD studied, compound **7** (a sulfonio-ammonium type of the thioxanthen-9-one dication) is characterized by the highest quantum yield of detritylation, $\varphi_{DMTr} = 0.59$. However, its synthesis is more complicated than the synthesis of APG **4**, which also possesses high quantum yield of detritylation, $\varphi_{DMTr} = 0.4$. Therefore, APG **4** was prepared on a large scale for the synthesis of oligonucleotides.

The data obtained can be used for the evaluation of the detritylation time of DMTrT on a biochip with APG **4** and intensity of the laser irradiation 10 mW mm^{-2} at the wavelength 375 nm, which can be achieved by the compact solid-state lasers. Let us accept the size of the biochip cell to be 10×10 μm, the number of cells 10^6 with the total working area 1 cm^2. The thickness of the polymer layer containing APG and DMTrT is 1 μm. Due to the high solubility of APG, its concentration in the polymer can reach 10^{-1} mol L^{-1}, the concentration of DMTrT is 10^{-4}—10^{-3} mol L^{-1} (see Ref. 12). When the laser irradiation with intensity I_0 is focused on the area 10×10 μm^2, the rate of photochemical reaction of acid generation for APG **4** is $\gamma I_0(1 - 10^{-(C d \varepsilon)}) = 0.4 \cdot 1.4 \cdot 10^3 (1 - 10^{-(10^{-1} \cdot 10^{-4} \cdot 300)}) =$ $= 3.85$ mol L^{-1} s^{-1}. Detritylation of 10^{-3} *M* DMTrT requires that the irradiation time was $2.6 \cdot 10^{-4}$ s, the full exposure time of the biochip without allowance for the time of the laser beam movement is 4.3 min.

The chosen APG **4** was tested in the synthesis of oligonucleotides in the DNA automated synthesizer modified for performing a photochemical reaction. A 1% solution of APG **4** was placed into the synthesizer reservoir instead of trichloroacetic acid for the removal of 5′-*O*-(4,4′-dimethoxytrityl) group. The following oligonucleotides were synthesized: dTTTTTTTTTT, dTACTGTCCTA, dATCCTTGGTC, and dAAAAAAAAAA. Amidites with the easily removable protecting groups were used for the elongation of the oligonucleotide chains: dT CEPA (5′-dimethoxytrityl-2′-deoxythymidine 3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite), (*N*-acetyl) dC CEPA (5′-dimethoxytrityl-*N*-acetyl-2′-deoxycytidine 3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite), (bz) dA CEPA (5′-dimethoxytrityl-*N*-benzoyl-2′-deoxyadenosine

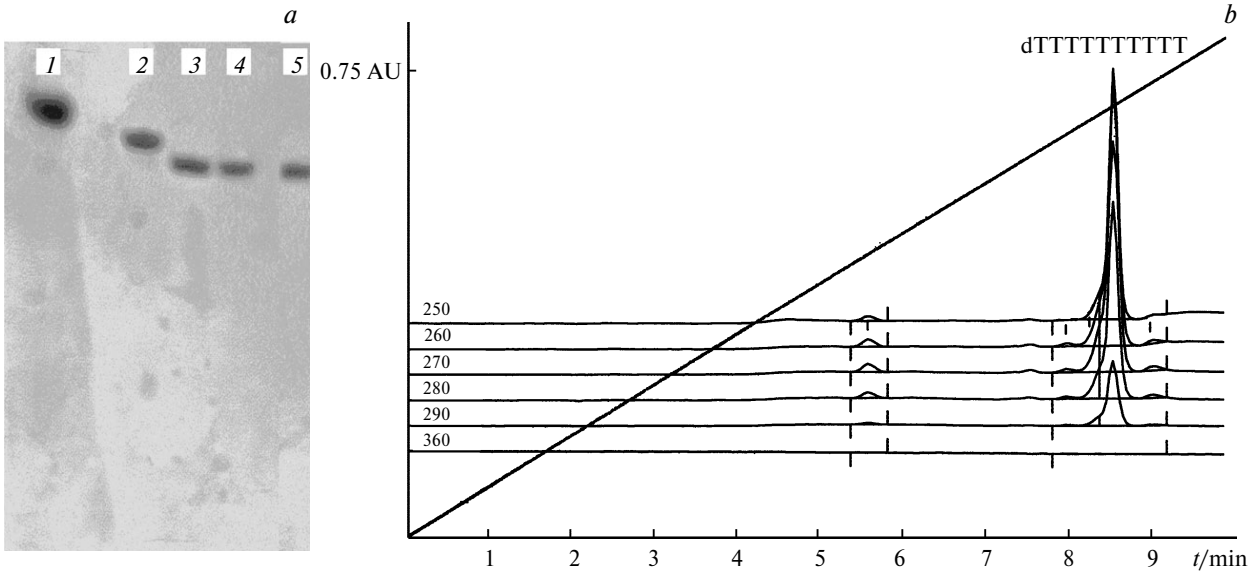

Fig. 9. (*a*) Electrophoregram in 15% PAAG (denaturing conditions): *1* is the reference (Bromophenol Blue), *2—5* are the reaction mixtures in the synthesis of dTTTTTTTTTT (*2*), dTACTGTCCTA (*3*), dATCCTTGGTC (*4*), dAAAAAAAAAA (*5*); (*b*) chromatography pattern obtained by the reversed-phase HPLC of the reaction mixture in the synthesis of dTTTTTTTTTT with monitoring at λ/nm (250—360 nm), AU are the optical units.

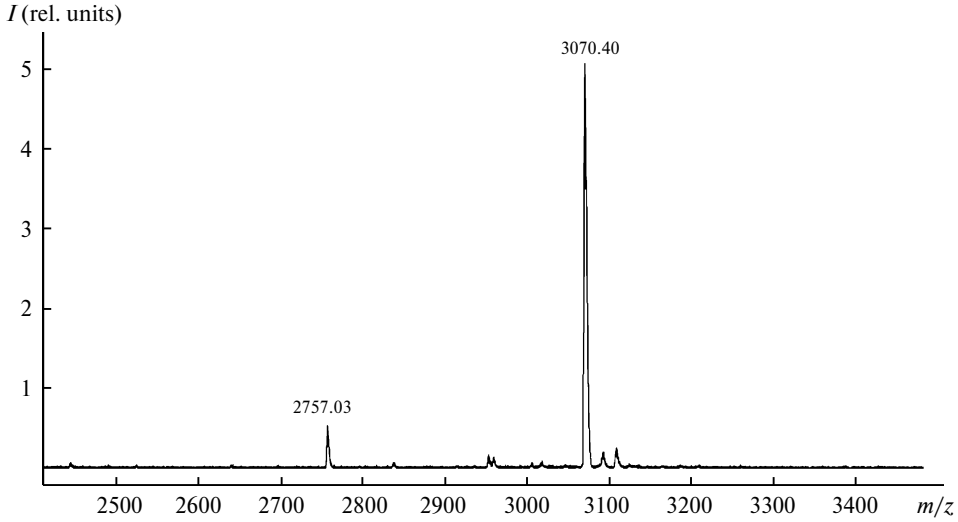

Fig. 10. Mass spectrum of dAAAAAAAAAA.

3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite), and (Ndmf) dG CEPA (5′-dimethoxytrityl-*N*-dimethylformamidine-2′-deoxyguanosine 3′-[(2-cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite). After completion of the synthesis, the support was treated with concentrated aqueous ammonia at 60 °C for 4 h, which released the target oligonucleotides from the solid phase and removed their protecting groups. The reaction mixtures after synthesis were analyzed by HPLC and gel-electrophoresis in PAAG (Fig. 9).

As follows from the analysis of the electrophoregrams (see Fig. 9, *a*, lanes *2—5*), the products with lower molecular weight are virtually absent, which indicates the efficient detritylation in the process of oligonucleotide synthesis. In addition, the absence of shorter oligonucleotides in the reaction mixture in the synthesis of dAAAAAAAAAA indicates the absence of apurinization in the process of oligonucleotide synthesis. This was additionally confirmed by mass spectrometry. The mass spectrum of dAAAAAAAAAA is given in Fig. 10, the molecular mass of the target product ion corresponded to the calculated one.

The synthesis of dTTTTTTTTTT was accomplished by two methods using dichloroacetic acid and APG **4** as detritylating agents. Spectroscopic characteristics of the oligonucleotide obtained by both methods corresponded to the literature data,[22] the A_{250}/A_{260} and A_{280}/A_{260} ratios were 0.66 and 0.69, respectively, which confirms its structure. The yields in each step of the chain elongation by both methods with the use of photogenerated or added acid in the synthesis of dTTTTTTTTTT are identical and equal to 98%.

In conclusion, we studied photochemical activity of a number of cationic thioxanthen-9-one derivative hexafluorophosphates in the detritylation reaction of 5′-*O*-(4,4′-dimethoxytrityl)thymidine, which resulted in the selection of compounds suitable for photodetritylation. Quantum yields were determined for the reactions of phototransformation of APG and photodetritylation of DMTrT. 10-(4-Heptyloxyphenyl)-9-oxo-2-(*N*,*N*,*N*-triethylammonio)methyl-9*H*-thioxanthenium bis(hexafluorophosphate) (**7**) proved the most efficient among the compounds studied, it possesses the quantum yield of photodetritylation 0.59 in dichloromethane on excitation with the light with $\lambda = 375$ nm. It is reasonable to carry out the photodetritylation reaction in dichloromethane.

The APG **4** was successfully used in the oligonucleotide synthesis in the step of the acid photogeneration, which makes it promising for the use in the microchip oligonucleotide synthesis.

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References

1. J. Cello, A. V. Paul, E. Wimmer, *Science*, 2002, **297**, 1016.
2. H. O. Smith, C. A. Hutchison, C. Pfannkoch, J. C. Venter, *Proc. Nat. Acad. Sci.*, 2003, **100**, 15440.
3. D. G. Gibson, J. I. Glass, C. Lartigue, V. N. Noskov, R. Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krish-

nakumar, N. Assad-Garcia, C. Andrews-Pfannkoch, E. A. Denisova, L. Young, Z. Q. Qi, T. H. Segall-Shapiro, C. H. Calvey, P. P. Parmar, C. A. Hutchison, H. O. Smith, J. C. Venter, *Science*, 2010, **329**, 52.
4. G. M. Church, *Scientific Am.*, 2006, No. 1, 47.
5. A. Y. Borovkov, A. V. Loskutov, M. D. Robida, K. M. Day, J. A. Cano, T. L. Olson, H. Patel, K. Brown, P. D. Hunter, K. F. Sykes, *Nucleic Acids Research*, 2010, **38**, No. 19, 180.
6. M. A. Russell, A. P. Laws, J. H. Atherton, M. I. Page, *Org. Biomol. Chem.*, 2009, **7**, 52.
7. L. I. McBride, M. H. Caruthers, *Tetrahedron Lett.*, 1983, **24**, 245.
8. S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett.*, 1981, **22**, 1859.
9. X. Gao, P. Yu, E. Le Proust, L. Sonigo, J. P. Pellois, H. Zhang, *J. Am. Chem. Soc.*, 1998, **120**, 12698.
10. X. Gao, E. Le Proust, H. Zhang, O. Srivannavit, E. Gulari, P. Yu, C. Nishiguchi, Q. Xiang, X. Zhou, *Nucleic Acids Res.*, 2001, **29**, 4744.
11. P. J. Serafinowski, P. B. Garland, *J. Am. Chem. Soc.*, 2003, **125**, 962.
12. P. J. Serafinowski, P. B. Garland, *Org. Biomol. Chem.*, 2008, **6**, 3284.
13. V. A. Loskutov, V. A. Shelkovnikov, *Zh. Org. Khim.*, 2006, **42**, 1113 [*Russ. J. Org. Chem.* (*Engl. Transl.*), 2006, **42**, 298].
14. V. A. Loskutov, V. A. Shelkovnikov, *Zh. Org. Khim.*, 2006, **42**, 313 [*Russ. J. Org. Chem.* (*Engl. Transl.*), 2006, **42**, 1097].
15. J. V. Crivello, *J. Pol. Sci.: Part A: Pol. Chem.*, 1999, **37**, 4241.
16. J. V. Crivello, J. H. W. Lam, *J. Pol. Sci.: Part A: Pol. Chem.*, 1996, **34**, 3231.
17. S. Mahajan, B. Vaijayanthi, G. Rembhotkar, K. C. Gupta, P. Kumar, *Methods Mol. Biol.*, 2007, **381**, 165.
18. M. A. Russell, A. P. Laws, J. H. Atherton, M. I. Page, *Org. Biomol. Chem.*, 2009, **7**, 52.
19. *Eksperimental´nye metody khimicheskoi kinetiki* [*Experimental Methods of Chemical Kinetics*], Eds N. M. Emanuel´, M. G. Kuz´min, MGU, Moscow, 1985, 384 pp. (in Russian).
20. G. M. Panchenkov, V. P. Lebedev, *Khimicheskaya kinetika i kataliz* [*Chemical Kinetics and Catalysis*], MGU, Moscow, 1961, 552 pp. (in Russian).
21. V. E. Kogan, G. S. Zenin, N. V. Penkina, *Fizicheskaya khimiya. Ch. 2. Khimicheskaya kinetika: Uchebnoe posobie* [*Physical Chemistry. Part 2. Chemical Kinetics: Textbook*], SZTU, SPb, 2005, 227 pp. (in Russian).
22. R. M. C. Dawson, D. C. Elliott, W. H. Elliott, K. M. Jones, *Data for Biochemical Research*, 3rd ed., Oxford Science Publications, OUP, Oxford, 1986, 580 pp.

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