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The Studies on the Synthesis and DNA Intercalative Properties of a New Type of Anthracene Probes Possessing Crown Ethers and Uracyl Groups

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A new procedure for the synthesis of aminoanthracene DNA probe **1** was developed. The model studies on the influence of protective groups on the reaction course towards formation of aminoanthracene **3a** were performed. The results of these studies were utilized in the synthesis of anthracene crown ether **10a**. Binding studies of this compound show low affinity ($K = 2.3\times10^{-1}$ M^{-1}) of this probe to CT-DNA. Smaller binding constant of this compound as compared to compound **3a** is presumably caused by complexation of sodium cation from the buffer. The model studies towards incorporation of uracyl group into aminoanthracene **10a** by formation of amide bonds were performed. The reaction proceeds only in the presence of *p*-nitrophenyl leaving group.

Key words: anthracene DNA probes, DNA intercalation, UV and fluorescence spectroscopy

During our studies towards synthesis of a new type of DNA intercalating agent we turned our attention on anthracene derivatives [1,2]. It has been pointed out that anthracene-shaped compounds intercalate without supporting positive charges with $\Delta G_i = 26$–27 kJ/mol [3]. The presence of supporting aminoalkyl substituents in the intercalating agents structure enhanced this intercalation by 5 kJ increment for each ion pairing. This was proved by anthracene functionalization. 9-Aminomethylanthracene (**1**) binds to natural and synthetic DNA with high affinity [4]. Increased electrostatic interaction, due to four positive N^+ groups present in compound **2** (described by Czarnik and Van Aman), also enhanced its intercalation to DNA [5]. This compound was prepared in reaction of 10-bis-chloromethyl-anthracene with 1,3-diaminopropane. The same approach to the synthesis of similar DNA probes was investigated also by Wunz [6]. Previously we have found that aminoanthracene intercalator **3a** binds to CT-DNA with high affinity [7]. The binding constant of this compound to CT-DNA, $K = 4.0 \times 10^4$ M^{-1}, was higher than that for aminoanthracene

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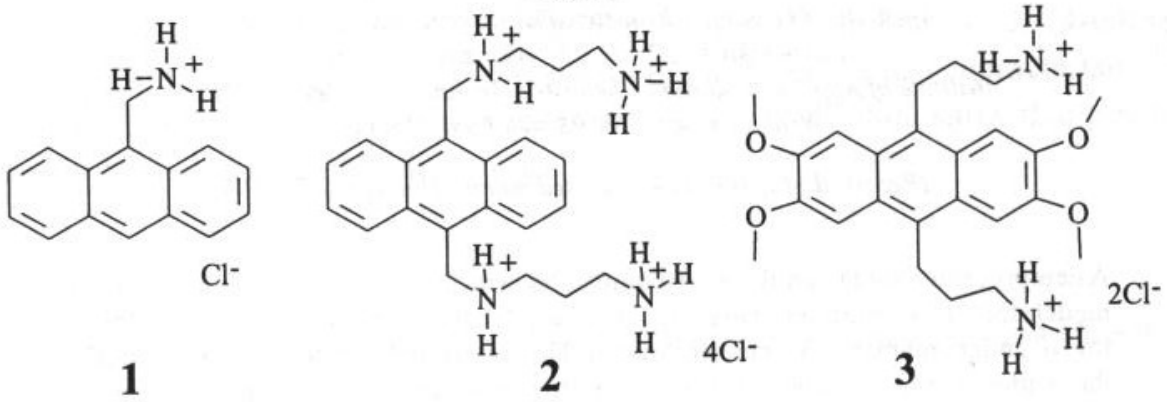
(**1**), $K = 1.0 \times 10^4\ M^{-1}$, already known from [4]. Since all experiments with DNA are performed in the NaCl buffer, incorporation of crown ether rings into the structure of probe **3** may enhance its water solubility and interactions with DNA.

This paper deals with the synthesis of a new type of anthracene probes, possessing crown ethers and uracyl groups and the studies of their interaction with CT-DNA.

Chart 1



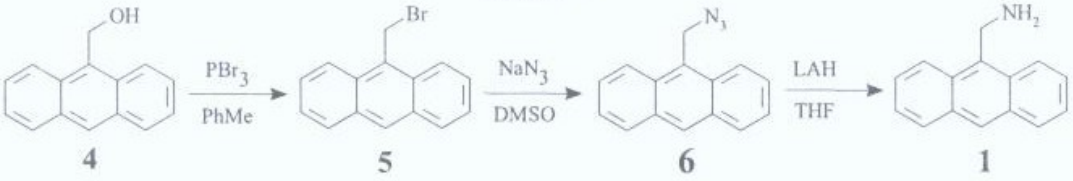
1 **2** **3**

RESULTS AND DISCUSSION

We intended to obtain aminoanthracene **1**, according to already known procedures, however, none of them was successful [4,8]. Therefore, the synthesis of 9-aminomethyl-anthracene **1** was based on the functionalization of alcohol **4**. This compound reacted with phosphorus tribromide in toluene to afford 9-bromomethyl-anthracene (**5**) in 77% yield. Reaction of bromide **5** with hexamethylenetetramine in chloroform did not lead to amine **1**, according to [9,10], but instead, 9-methyl-anthracene was obtained in over 90% yield. Reaction of bromide **5** with sodium azide in DMSO at 50°C afforded 90% of 9-azidomethyl-anthracene (**6**). This reaction proceeds smoothly below 60°C. At higher temperature, formation of a side product was observed. Reduction of the azido group can be performed with $LiAlH_4$ in THF at 30°C or $NaBH_4$ in toluene–methanol mixture at 10°C. In both cases, the yield of pure amine **6** was almost the same (88%), but reduction with $NaBH_4$ was more convenient. Treatment of free amine **1** in toluene with gaseous HCl precipitated the hydrochloride. Purification of this compound by crystallization failed, since formation of respective cyclodimers was noted above 40°C. This cyclodimer was characterized previously [8]. Therefore, for DNA binding experiments amine **1** was purified by precipitation from water– methanol solution.

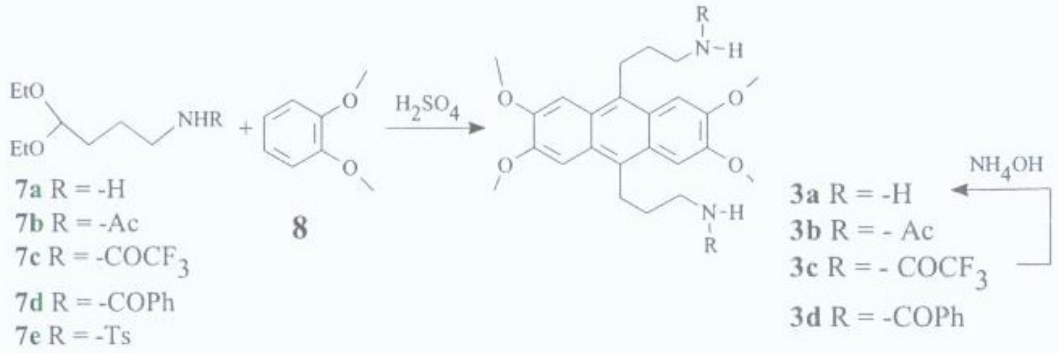
Previously we have published the synthetic procedure for the preparation of compound **3** [7], based on modification of the literature procedure [10].

Scheme 1



4 **5** **6** **1**

Scheme 2



7a R = -H
7b R = -Ac
7c R = -$COCF_3$
7d R = -COPh
7e R = -Ts

8

3a R = -H
3b R = - Ac
3c R = - $COCF_3$
3d R = -COPh

The synthesis based on unprotected aminoacetal **7a** was found to be hardly reproducible. Therefore, we decided to protect amino groups in order to improve the yield of reaction and simplify the purification of the products desired. In the first experiment, the acetyl group was used. Desired amide **7b** was obtained upon mixing of amine **7a** with ethyl acetate in 95% yield [11]. This compound was used for the next reaction, but only traces of **3b** were obtained and attempted purification failed, due to insolubility of the product. Application of trifluoroacetyl group [12] was more successful. Reaction of acetal **7c** (prepared from trifluoroethyl acetate and amine **7a** according to [13] in 95% yield) and veratrole gave bisamide **3c** in 45% yield. Deprotection of amine functions can be performed upon treatment with NH_4OH solution, according to [14]. Unfortunately, low solubility of amide **3c** forced us to modify the reaction conditions. We have found that the desired compound can be obtained in dioxane containing 5% NH_4OH at 10°C. At higher temperature, decomposition of **3a** was observed and, finally, the expected product was obtained in 33% yield after crystallization. For DNA binding studies, hydrochloride of bisamine **3a** was obtained by treatment of amine solution with HCl. Protection of the amino group in compound **3a** with benzoyl [15] and tosyl [16] groups in compounds **7d** and **7e,** respectively, did not allow to prepare the desired compounds of structure **3e**. These studies on the influence of protective groups on the yield of reaction between N-protected aminoacetals **7** and veratrole were used for the synthesis of bisanthracene crown ethers **10a**.

Reaction of benzo-15-crown-5 (**9**) with amine **7a** in the presence of sulphuric acid led to anthracene crown **10a** in 19% yield. Application of amide **7b** allowed to prepare desired compound **10b** in 35 % yield. Unfortunately, poor solubility of this compound limited its reactivity, what was observed upon deprotection of amido functions. Reaction with ammonium hydroxide proceeded slowly and the desired compound **10a** was ob-

(**1**), $K = 1.0 \times 10^4\ M^{-1}$, already known from [4]. Since all experiments with DNA are performed in the NaCl buffer, incorporation of crown ether rings into the structure of probe **3** may enhance its water solubility and interactions with DNA.

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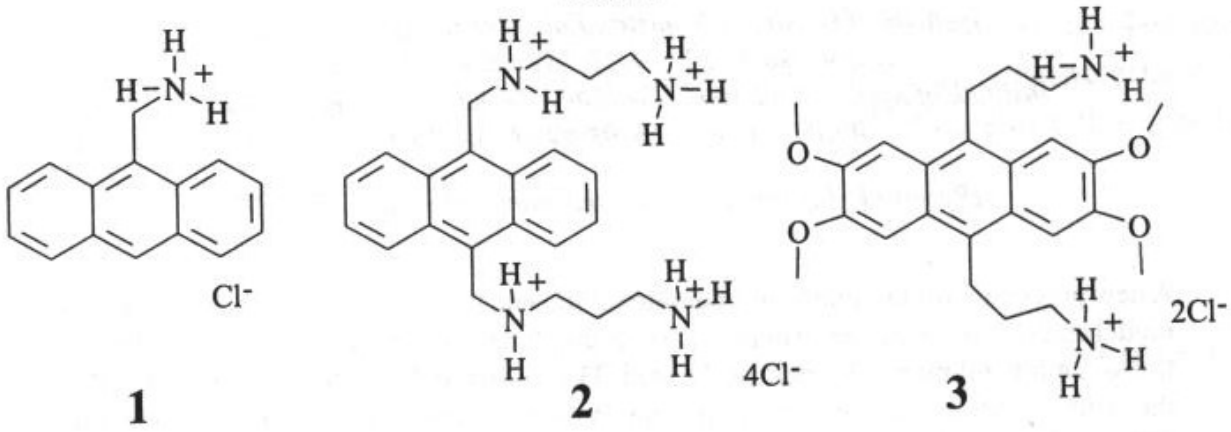

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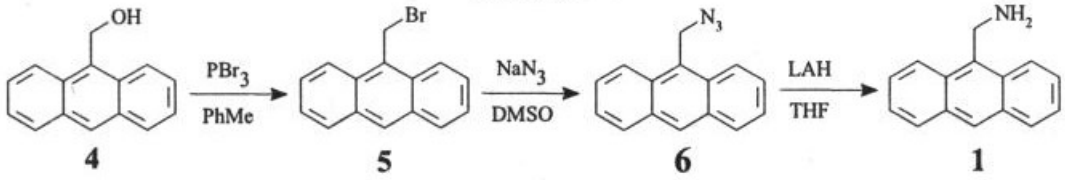
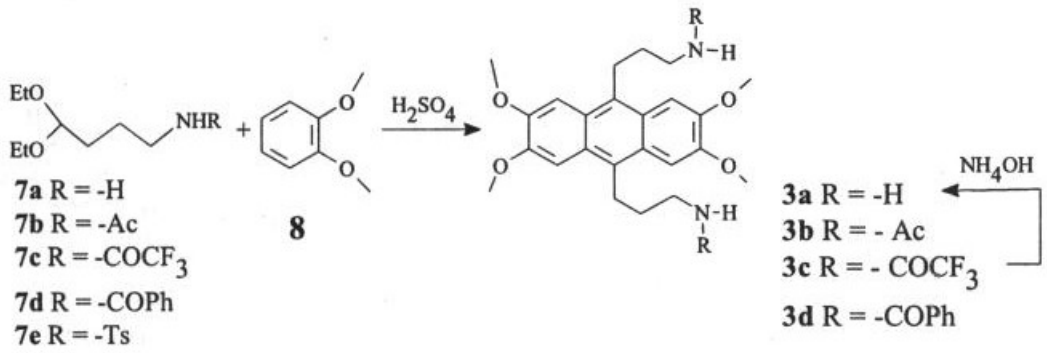


Scheme 2



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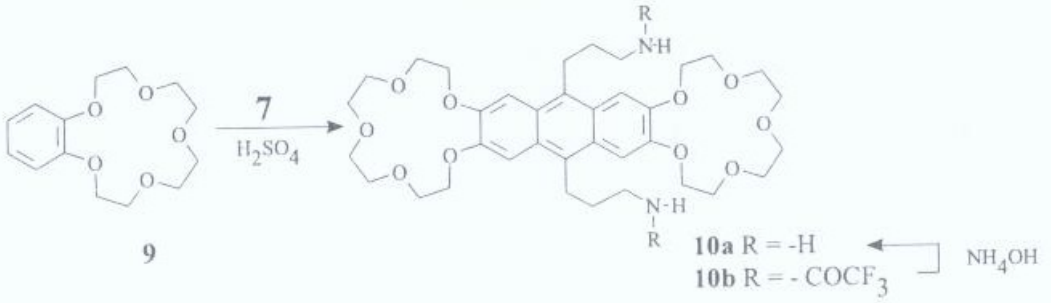
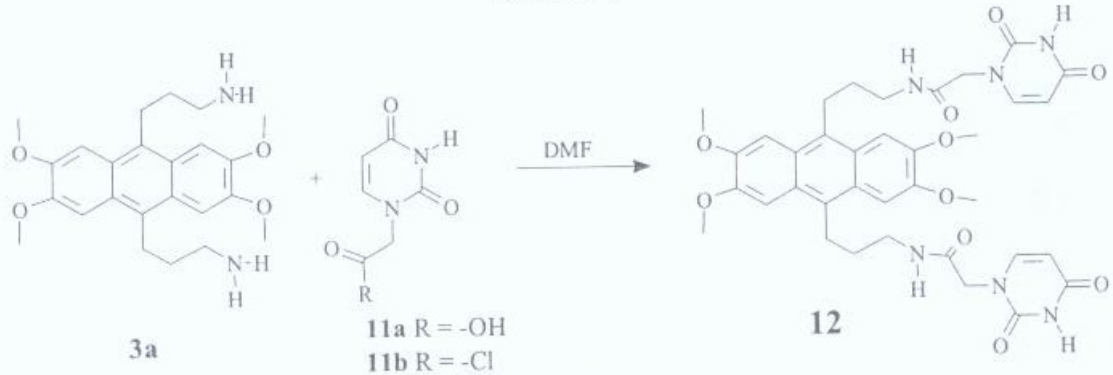


Scheme 4



tained in 25% yield after recrystallization from toluene. The hydrochloride of amine **10a**, required for DNA binding studies, was obtained in the usual way.

The interaction of anthracene amines to DNA can be enhanced by uracyl groups in the ligand structure, capable to interact with DNA by formation of hydrogen bonds to DNA bases. As a target structure, easily accessible uracyl derivative **11a** was selected. This compound was prepared, according to procedure in 65% yield [17]. Its reactivity towards formation of amide bond to compound **3a** was studied. Direct coupling procedure, using DCC or DCC-HOBT, failed [18] and none of the desired compounds was obtained. In the next step, the respective acyl chloride **11b** was prepared [19]. Unfortunately, this compound did not react with amine **3a** and amide **12** was not formed. In the next step, *p*-nitrophenyl ester **11c**, derived from acid **11a**, was prepared [20]. Its reaction with anthracene amine **3a** proceeded in 91% yield and compound **12** was obtained in DMF at 50°C. The target amide obtained is poorly soluble in protic and aprotic solvents and therefore its interaction with CT-DNA cannot be studied. Similar problems with the solubility of the target compounds were already mentioned [21].

DNA binding studies. For the DNA binding experiments only compounds of good solubilities in water can be used. Our previous experiments were performed on probes **1** and **3a**. Since incorporation of the uracyl group into structure **12** decreased its solubility in water, binding studies of compound **10a** to CT-DNA were performed, according to previously used methods [7], using absorption and fluorescence spectroscopy. In the absorption spectra of the target compounds, increasing the amount of CT-DNA caused

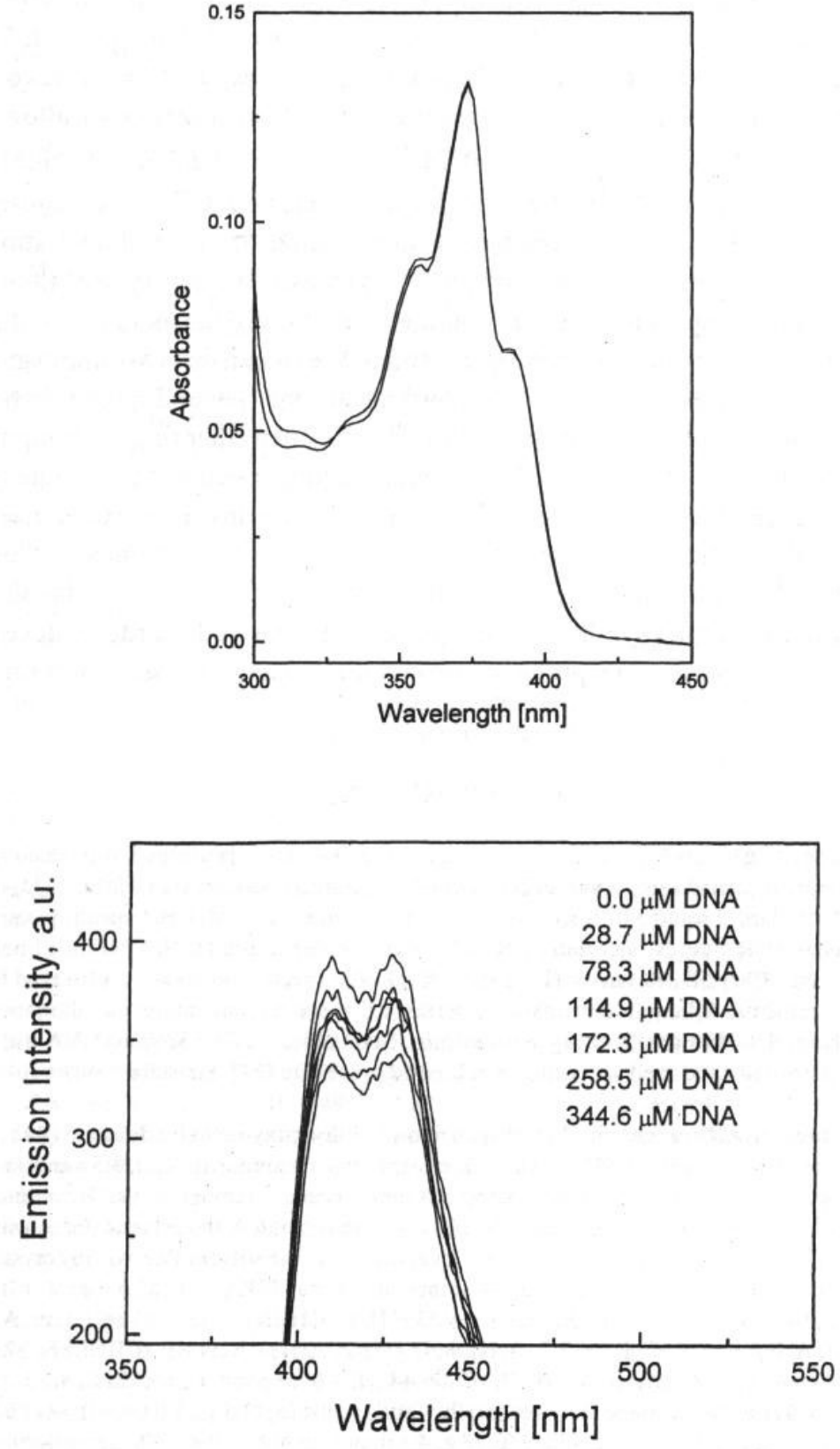


Figure 1. The changes in UV (upper) and fluorescence spectra of compound **3a** (54.3 μM of mmol dm^{-3}) upon addition of CT DNA (in aqueous 50 mM NaCl, at pH = 7.0).

only a small decrease in the peak intensities (hypochromicity), small broadening and a red shift in UV spectra (Figure 1). Fluorescence studies proved, that upon addition of the CT-DNA to the solution of **10a** quenching of fluorescence by DNA bases was observed. Analysis of the fluorescence data, according to Stern-Volmer equation [4], allowed to estimate the binding constant at $K = 2.3 \times 10^1\ M^{-1}$, which is smaller than the value obtained for probe **3a** ($K = 4.0 \times 10^4\ M^{-1}$). Weak binding of our probe to CT-DNA is caused by the crown ether rings in the probe structure. Complexation of two sodium cations from buffer weakens the electrostatic interaction [3]. Synthesis of a new type of fluorescence probes, based on a simple molecule of veratrole type, allowed the monitoring of its interaction with CT-DNA by the spectroscopic method. We found, that two amino groups enhance the intercalation with DNA, according to the general probe behaviour. Modification of the probe **3a** by incorporation of two crown ether rings in compound **10a** diminished its interaction with CT-DNA. Therefore, the presence of crown ethers ring in DNA probes is highly untidy. Incorporation of uracyl group in probe structure, *via* amide bonds, resulted in a strong decrease of the solubility of these compounds. Although, the presence of such a group in the probe structure can strongly influence its interaction with DNA [22,23], further synthetic methodologies are required in order to develop suitable procedures for anthracene probe uracyl coupling reactions, based on formation on C–N bonds.

EXPERIMENTAL

General. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Varian 200 Gemini spectrometer in $CDCl_3$ or $CDCl_3/C_6D_6$ with TMS as an internal standard. Liquid SIMS spectra were determined on an AMD 604 spectrometer (Cs^+, 10 keV). The steady-state fluorescence and excitation spectra were recorded with a Perkin-Elmer LS-50B Spectrofluorimeter. Cary 5E (Varian) or Hewlett-Packard 8452 spectrophotometers were used for the absorption measurements. All measurements were performed at room temperature, and the samples were deaerated by careful bubbling with nitrogen for 20 min. Sodium salt of Calf Thymus DNA was obtained from Merck, Darmstadt, and used according to published procedure [24]. Phosphate buffer (pH = 7) was purchased from POCh, Gliwice.

9-Bromomethyl-anthracene 5: The suspension of 9-hydroxymethylanthracene (**4**) (1.5 g, 7.2 mmol) in toluene (40 ml) cooled to 0°C in ice-bath, phosphorus tribromide (0.8 ml, 8.5 mmol) was added and the reaction mixture was left for 1 hour; during this time it became homogeneous. Then, saturated sodium carbonate solution (10 ml) was added, the phases were separated, the organic phase was washed with water (5 ml), brine (5 ml) and dried ($MgSO_4$). Evaporation of the solvent followed by crystallization of the residue from toluene gave 9-bromomethyl-anthracene (1.5 g, 77% yield). M.p. 135.2–138.0°C (toluene) (lit. [12] 140–142°C). ^{1}H NMR ($CDCl_3$) δ: 5.53 (2H, s, -C**H**$_2$Br), 7.45–7.68 (4H, t, m, Ar**H**), 8.03 (2H, d, J = 8.4, Ar**H**), 8.30 (2H, d, J = 8.7, Ar**H**), 8.47 (1H, s, Ar**H**). ^{13}C NMR ($CDCl_3$) δ: 27.6, 125.9, 127.3, 129.7, 129.8. Anal. Calcd. for $C_{15}H_{11}Br$: C, 66.44; H, 4.09. Found: C, 66.02; H, 4.50.

9-Azidomethyl-anthracene 6: 9-Bromomethyl-anthracene (**5**) (1.1 g, 4.0 mmol) was dissolved in DMSO (25 ml) and sodium azide was added (0.27 g, 4.1 mmol) in one portion. The temperature of reaction mixture was raised to 50°C and stirring was continued at this temperature for 4 h. Then, reaction mixture was cooled to room temperature and water (40 ml) was added, followed by ethyl acetate (20 ml). The phases were separated and the aqueous one was extracted by ethyl acetate (2 × 20 ml). The organic phases were combined, washed with brine (15 ml) and dried ($MgSO_4$). The solvent was evaporated and the crude product was purified by crystallization from toluene (1.35 g, 90% yield). M.p. 82.3–83.1°C. ^{1}H NMR ($CDCl_3$) δ: 5.26 (2H, s, -C**H**$_2$N$_3$), 7.42–7.62 (4H, t, m, Ar**H**), 8.00 (2H, br d, J = 8.2, Ar**H**), 8.25 (2H, br d,

J = 9.0, Ar**H**), 8.45 (1H, s, Ar**H**). ^{13}C NMR ($CDCl_3$) δ: 46.2, 123.4, 125.1, 126.7, 128.9, 129.2, 130.6, 131.3. Anal. Calcd. for $C_{15}H_{11}N_3$: C, 77.23; H, 7.75, N, 18.01. Found: C, 77.04; H, 4.58; N, 17.98.

9-Aminomethyl-anthracene 1: 9-Azidomethyl-anthracene (**6**) (1.2 g, 3.2 mmol) was dissolved in ethanol (40 ml) and sodium borohydride was added in two portions (0.12 g, 3.2 mmol) keeping the temperature of reaction mixture below 5°C. After 20 minutes, the cooling bath was removed and the reaction mixture was left for 1 hour. The excess of reducing reagent was decomposed by addition of hydrochloric acid, the solvent was evaporated, the residue was dissolved in water (40 ml) and the aqueous phase was extracted with toluene (2 × 10 ml). Water phase was adjusted to pH 10 by addition of 5% NaOH and the product was extracted with toluene (2 × 20 ml). The organic phases were combined, washed with brine (15 ml), dried ($MgSO_4$), and the crude product was crystallized from toluene: m.p. 98.5–101.0°C (lit. [25] 98–100°C). Hydrochloride of **1** was precipitated from the chloroform solution of the amine by treatment with gaseous HCl. ^{1}H NMR (DMSO-d_6) δ: 5.05 (2H, d, J = 5.2, -C**H**$_2$NH$_3$), 7.48–7.69 (4H, m, Ar**H**), 8.18 (2H, d, J = 8.1, Ar**H**), 8.45 (2H, d, J = 8.5, Ar**H**), 8.76 (1H, s, Ar**H**).

Synthesis of 2,3,6,7-tetramethoxy anthracene probes. General synthetic procedure: To the intensively stirred, cooled to 5°C (ice bath) solution of 84% sulphuric acid (10 ml), a solution of the respective N-protected aldehyde **3** (10 mmol), veratrole (0.28 g, 2 mmol) or benzo-15-crown-15 (0.536 g, 2 mmol) in chloroform (5 ml) was added slowly, while keeping the temperature below 10°C. The reaction mixture became dark red and was stirred at this temperature for 1 h, then the cooling bath was removed and stirring was continued until TLC indicated complete conversion of the benzo-15-crown-5 (usually 1 h). Water (50 ml) was added followed by ammonium hydroxide to reach pH 11. Aqueous phase was extracted with chloroform (3x 30 ml) and the organic phases were combined and dried ($MgSO_4$). In most cases, pure products were purified by column chromatography on silica gel using chloroform–methanol solvent mixture (95/5, v/v).

9,10-Di(3-aminopropyl)-2,3,6,7-tetramethoxy-anthracene 3a: Crude amine was purified by recrystallization from toluene. Hydrochloride **3** was precipitated from the chloroform solution after treatment of the amine with gaseous HCl. Overall yield 55%. ^{1}H NMR (DMSO-d_6) δ: 1.99 (4H, br s, -C**H**$_2$CH$_2$N), 3.10 (4H, t, J = 6.8 Hz, -CH$_2$C**H**$_2$N), 3.46–3.58 (4H, m, ArC**H**$_2$-), 4.02 (18H, br s, -OC**H**$_3$ + N**H**$_3$), 7.41 (4H, s, Ar**H**). ^{13}C NMR ($CDCl_3$) δ: 25.3, 28.2, 56.1, 96.0, 102.9, 125.5, 128.4, 149.6. Anal. Calcd. for $C_{24}H_{34}O_4Cl_2$+H_2O: C, 57.26; H, 7.21; N, 5.56. Found: C, 57.02; H 7.50; N, 5.34.

9,10-Di(3-(trifluoracetylamino)propyl)-2,3,6,7-tetramethoxy-anthracene 3c: Crude amide precipitated upon dilution of reaction mixture with methanol was filtered off and crystallized from chloroform. Yield 45%. ^{1}H NMR (DMSO-d_6) δ: 1.96 (4H, m, -C**H**$_2$CH$_2$N), 3.45–3.55 (8H, m, -C**H**$_2$CH$_2$C**H**$_2$N), 4.01 (12H, s, -OC**H**$_3$), 7.41 (4H, s, Ar**H**), 9.66 (s, 2H, NH). ^{13}C NMR ($CDCl_3$) δ: 25.6, 29.8, 55.5, 96.0, 102.5, 125.1, 128.2, 149.1, 173.4. IR (KBr): 3308*m* (NH), 1704*vs* (C=O), 1567*m*, 1500*s*, 1436*m*, 1248*s*, 1204*s*, 1179*s*, 1034*m*, 827*w*, 525*w*. LSIMS (NBA) m/z 604 ([M+H]$^+$, 100%), 460 (25%). HR-LSIMS *m/z* 604.2039 (604.2009 calcd. for $C_{28}H_{30}O_4N_2F_6$, [M]$^+$). Anal. Calcd. for $C_{28}H_{30}O_4N_2F_6$+$CHCl_3$: C, 48.19; H, 4.33; N, 3.33. Found: C, 48.26; H, 4.49; N, 3.88.

Deprotection of the amino groups in compound 3c. To the suspension of bisamide **3c** (0.30 g, 0.41 mmol) in dioxane (100 ml), cooled to 10°C, ammonium hydroxide (1 ml, 25% in water) was added, the reaction mixture was stirred at this temperature for 8 h and concentrated. Crude product was purified by recrystallization from toluene. The hydrochloride obtained from this bisamine in overall 33% yield was identical with **3a** prepared previously.

9,10-Di(3-aminopropyl)-2,3-6,7-di(15-crown-5)-anthracene 10a: Crude amine was purified by recrystallization from toluene. Hydrochloride **3** was precipitated from the chloroform solution after treatment of the amine with gaseous HCl. Overall yield 55%. ^{1}H NMR (DMSO-d_6) δ: 1.99 (4H, br s, -C**H**$_2$CH$_2$N), 3.10 (4H, t, J = 6.8 Hz, -CH$_2$C**H**$_2$N), 3.46–3.58 (4H, m, ArC**H**$_2$-), 4.02 (18H, br s, -OC**H**$_3$ + N**H**$_3$), 7.41 (4H, s, Ar**H**). ^{13}C NMR ($CDCl_3$) δ: 25.3, 28.2, 56.1, 96.0, 102.9, 125.5, 128.4, 149.6. Anal. Calcd. for $C_{24}H_{34}O_4Cl_2$+H_2O: C, 57.26; H, 7.21; N, 5.56. Found: C, 57.02; H 7.50; N, 5.34.

9,10-Di(3-(trifluoroacetylamino)propyl)-2,3-6,7-di(15-crown-5)-anthracene 10b: Crude amide precipitated upon dilution of reaction mixture with methanol was filtered off and crystallized from chloroform. Yield 35%. ^{1}H NMR (DMSO-d_6) δ: 1.89 (4H, br s, -C**H**$_2$CH$_2$N), 3.30–3.49 (8H, m, -C**H**$_2$CH$_2$C**H**$_2$N), 3.67 (16H, br s, -OC**H**$_2$C**H**$_2$-), 3.88 (8H, br s, ArOC**H**$_2$C**H**$_2$O-), 4.24 (8H, br s, ArOC**H**$_2$CH$_2$O-), 7.36 (4H, s, Ar**H**), 9.61 (2H, s, NH). IR (KBr): 3301*m* (NH), 1706*vs* (C=O), 1569*m*, 1499*vs*, 1451*m*, 1250*s*, 1185*vs*, 1158*s*, 1088*m*, 1034*m*, 984*m*, 632*w*, 889*w*, 723*w*. LSIMS (NBA) *m/z* 887

([M+Na]$^+$, 100%), 864 ([M]$^+$, 18%). Anal. Calcd. for $C_{40}H_{50}O_{12}N_2F_6$+$CHCl_3$ C, 50.39; H, 5.22; N, 2.85. Found: C, 50.39; H 5.38; N, 2.77.

Deprotection of amino groups in compound 10b. To the suspension of bisamide **3c** (0.25 g, 0.25 mmol) in dioxane (100 ml), cooled to 10°C, ammonium hydroxide (1 ml, 25% in water) was added and reaction mixture was stirred at this temperature for 8 h. Then, the solvents were evaporated and the crude product was purified by recrystallization from toluene. The hydrochloride obtained from this bisamine in overall 25% yield was identical with **10a** prepared previously.

Synthesis of uracyl - anthracene probe 12. To a stirred solution of amine **3a** (0.24 g, 0.5 mmol) in DMF (10 ml), a solution of uracyl derivative **11c** (0.32 g, 1.1 mmol) in DMF (5 ml) was added at room temperature and precipitation of product was observed within 5 minutes. The reaction mixture was heated at 50°C for 0.5 hour, cooled to room temperature, and then methanol was added (25 ml). The precipitated product was filtered off, washed with methanol and dried under vacuum. Yield 91%. ^{1}H NMR (DMSO-d_6) δ: 1.90 (4H, br s, -C**H**$_2$CH$_2$N), 2.56 (4H, br s, COC**H**$_2$N), 3.20–3.41 (8H, m, -CH$_2$CH$_2$C**H**$_2$N), 3.46 (12H, br s, -OC**H**$_3$), 5.56 (2H, br d, J = 7.9, NC**H**=CH-), 6.60 (2H, s, CON**H**), 7.43 (4H, s, Ar**H**), 7.55 (2H, br d, J = 7.9, NCH=C**H**-), 11.31 (2H, s, N_{urac}**H**); IR (KBr): 3418*m* (N–H), 3303*m* (N–H intermolecular), 1684*b vs* (C=O), 1567*m*, 1500*m*, 1455*m*, 1352*s*, 1249*s*, 1106*s*, 942w, 805*w*, 553*w*. LSIMS (NBA) *m/z* 717 ([M+H]$^+$, 1.5%), 605 ([M-uracyl]$^+$, 11%), 111 (100%); Anal. Calcd. for $C_{36}H_{40}O_{10}N_6$: C, 60.36; H, 5.63; N, 11.73. Found: C, 60.79; H 5.38; N, 11.27.

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