Nucleotides

Part LXXVI[1])

Synthesis and Properties of New Isoalloxazine (= Benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) Derivatives as Labels for Oligonucleotides

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A series of new fluorescing 8-(6-hydroxyhexyl)isoalloxazine (= 8-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) derivatives **4**–**13** were synthesized from 6-[(6-hydroxyhexyl)amino]uracil (**2**) with 1-chloro-4-nitrosobenzene *via* 8-chloro-10-(6-hydroxyhexyl)isoalloxazine (**3**) and subsequent substitution of the Cl-atom of **3** by various amines (*Scheme*). Analogously, 8-substituted 10-{3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}isoalloxazines **19**, **20**, and **23**–**25** were prepared which yielded on deprotection the corresponding 10-[3-(2,3-dihydroxypropoxy)propyl]alloxazines **21**, **22**, and **26**–**28**. Their conversion into the 3′′-*O*-(4,4′-dimethoxytrityl) derivatives **29**–**33** and subsequent transformation into the corresponding 2′′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidites) **34**–**38** led to new building blocks for oligonucleotide synthesis. A series of 21-mer oligodeoxyribonucleotides carrying the fluorescing isoalloxazine **37** in various positions of the chain were assembled in a DNA synthesizer. Combination with the complementary sequence yielded the stable duplexes **40**–**54** showing by the melting temperatures T_m that the fluorophor (**F**) does not harm the stability of the unmodified duplex **39** (*Table*).

Introduction. – Labeling of biomolecules can be regarded as the most common and widely used methodology for a broad variety of bioanalytical purposes. Fluorescence labeling [2], especially, is often the preferred choice due to the high sensitivity and easy detection. We concentrated our interest on isoalloxazine (= benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) derivatives which show strong fluorescence, high quantum yields, and high extinctions in the VIS region of 460–480 nm. The best-known isoalloxazine derivative is vitamin B_2 which is produced technically on a large scale by the classical method condensing N^1-D-ribityl-4,5-dimethylbenzene-1,2-diamine with alloxan [3][4].

Alloxazine syntheses are more versatile and include the condensation of an *o*-aminoazobenzene with a barbituric acid [5] or a 5-nitrosopyrimidine with an aromatic amine [6], or the nitrosation of a 6-(arylamino)uracil [7]. Further possibilities are seen in the reactions of 6-anilinouracils with diethyl azodicarboxylate [8][9] or by refluxing 1,3-dimethyluracil-6-amine with nitrosobenzenes in Ac_2O [10]. Our approach to various 10-substituted isoalloxazines was based [11] on the condensations of 6-(alkylamino)uracils with 1-chloro-4-nitrosobenzene in DMF leading to 8-chloro-10-alkylisoalloxazines which could further been modified at position 8 by nucleophilic substitution reactions.

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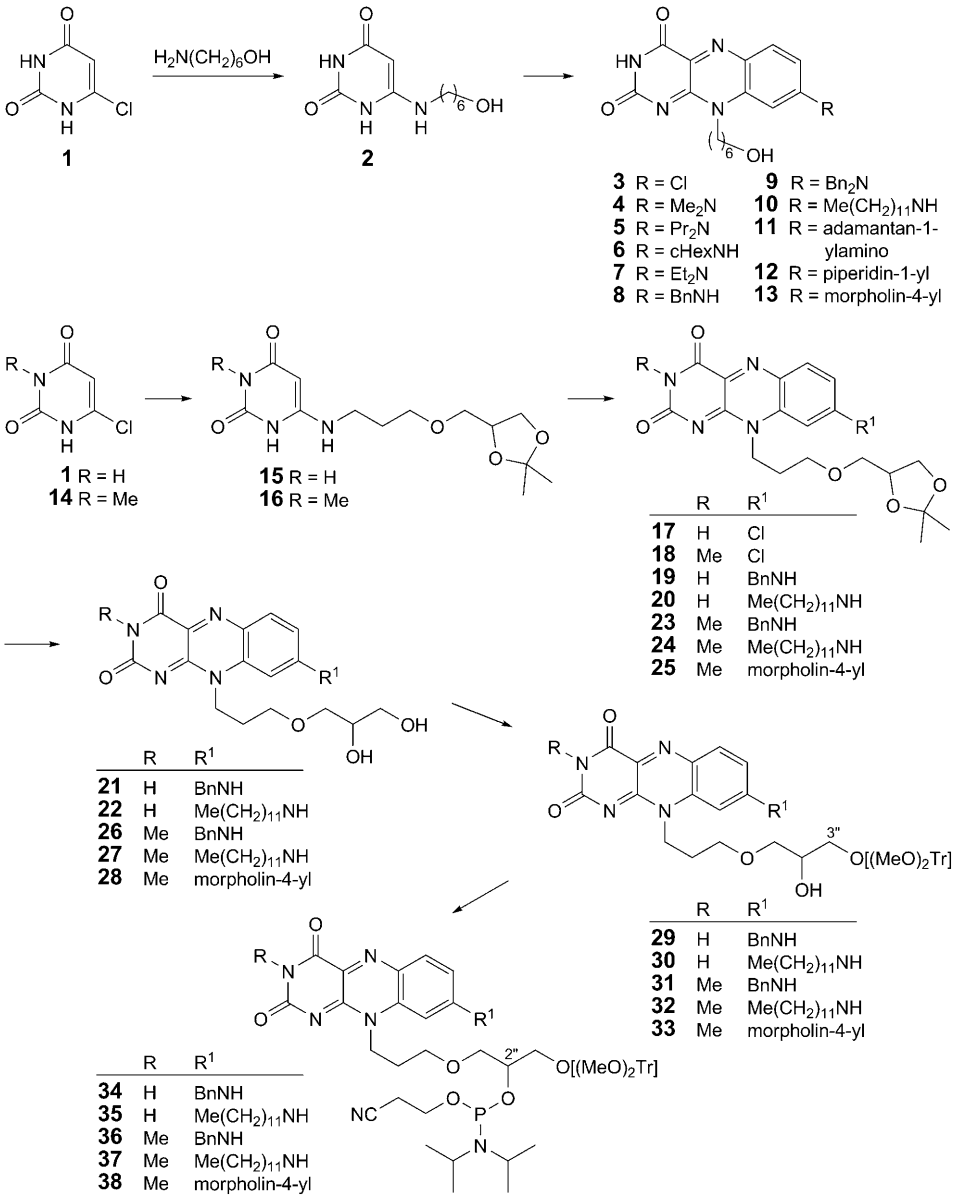
Synthesis. – Starting from 6-chlorouracil (**1**), reaction with 6-aminohexan-1-ol gave 6-[(6-hydroxyhexyl)amino]uracil (**2**) which could be converted with 1-chloro-4-nitrosobenzene in DMF directly in high yield into 8-chloro-10-(6-hydroxyhexyl)isoalloxazine (**3**) (*Scheme*). In a similar manner reacted *N*,*N*-dimethyl-4-nitrosobenzenamine to the corresponding 8-(dimethylamino)-10-(6-hydroxyhexyl)isoalloxazine (**4**), and in

Scheme



a stepwise reaction, **2** was first converted with 1-chloro-4-nitrosobenzene into **3** followed by treatment with Pr_2NH or cyclohexanamine to give **5** and **6**, respectively. A more convenient approach was the nucleophilic substitution of the Cl-substituent in **3** by Et_2NH, benzylamine, *N*,*N*-dibenzylamine, dodecan-1-amine, adamantan-1-amine (= tricyclo[3.3.1.1^{3,7}]decan-1-amine), piperidine, and morpholine yielding **7**–**13**.

To prepare appropriate building blocks for oligonucleotide synthesis, **1** and 6-chloro-3-methyluracil (**14**) were treated with 3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propan-1-amine to form **15** and **16**, respectively. Condensation of **15** with 1-chloro-4-nitrosobenzene in *N*,*N*-dimethylacetamide (DMA) yielded 8-chloro-10-{3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}isoalloxazine (**17**) which was further transformed with benzylamine or dodecan-1-amine into **19** and **20**, respectively. The deprotection of the side chain at N(10) of **19** and **20** was achieved in 80% AcOH to give 8-(benzylamino)- (**21**) and 10-[3-(2,3-dihydroxypropoxy)propyl]-8-(dodecylamino)-isoalloxazine (**22**). The corresponding 3-methyl derivatives **26** and **27** as well as the 8-(morpholin-4-yl) compound **28** resulted from a one-pot reaction starting with **16** which was converted to **23**–**25** *via* **18** and, without isolation, directly deblocked to **26**–**28**. The deprotected 8-substituted 10-[3-(2,3-dihydroxypropoxy)propyl]isoalloxazines **21**, **22**, and **26**–**28** were then treated with 4,4′-dimethoxytrityl chloride to give **29**–**33**. The latter were transformed with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite under 1*H*-tetrazole catalysis to the corresponding phosphoramidites **34**–**38** in good yields (*Scheme*).

Oligonucleotides. – To test the new isoalloxazine derivatives as fluorescence markers, a series of oligodeoxyribonucleotides with the fluorophor in various positions of the chain were synthesized by well-known procedures in an *ABI* synthesizer *A392*. As solid support was used *LCAMA-CPG* 500 Å [12–14] to which 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-succinylthymidine was attached first. The chosen 21-mer sequence was 5′-d(GTG TGG AAA ATC TCT AGC AGT)-3′ which showed with its complementary sequence 3′-d(CAC ACC TTT TAG AGA TCG TCA)-5′ a melting temperature of 52.7° (see **39** in the *Table*). The fluorescing phosphoramidite **36** (*cf.* **F**) was built into the chain from the 5′-end successively in positions 1–6, 8, 10, 11, 13, 15, and 19 (*Table*) providing the duplexes **40**–**54** with melting temperatures which were higher in all cases than that of the unmodified duplex **39** (*Table*). In duplexes **49**–**52**, position 13 in the complementary chain was also modified by the four natural bases showing, however, no special effect on the melting temperature.

The monomeric building blocks for the oligodeoxyribonucleotide synthesis were the 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^6-{[2-(4-nitrophenyl)ethoxy]carbonyl}adenosine, 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-N^4-{[2-(4-nitrophenyl)ethoxy]carbonyl}-cytidine, and 2′-deoxy-5′-*O*-(dimethoxytrityl)-N^2-{[2-(4-nitrophenyl)ethoxy]carbonyl}-O^6-[2-(4-nitrophenyl)ethyl]guanosine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) [15]. Deprotection at the nucleobases was achieved by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) treatment, a washing process, and finally cleavage from the support by ammonia [14]. The oligomers were of high purity as seen from HPLC analysis on reversed phase *RP-18* material.

Table. *Melting Temperatures and Fluorescence Intensities of Modified Oligonucleotides* **40**–**54**[a])

	Sequence	T_m [°]	Fluorescence intensity at	
			25°	65–75°
39	5′-d (GTG TGG AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	52.7		
40	5′-d (**F**TG TGG AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	62.5	17	10.5
41	5′-d (G**F**G TGG AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	59.5	22	12
42	5′-d (GT**F** TGG AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	57.3	1.6	1.5
43	5′-d (GTG **F**GG AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	56.5	1.0	1.4
44	5′-d (GTG T**F**G AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	55.3	4.8	3.4
45	5′-d (GTG TG**F** AAA ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	53.5	7.4	5.7
46	5′-d (GTG TGG A**F**A ATC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	54.8	16.4	10.6
47	5′-d (GTG TGG AAA **F**TC TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	53.5	28.8	21.5
48	5′-d (GTG TGG AAA A**F**C TCT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	54.2	11	14
49	5′-d (GTG TGG AAA ATC **F**CT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG **A**GA TCG TCA)-5′	55.8	23.5	31
50	5′-d (GTG TGG AAA ATC **F**CT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG **C**GA TCG TCA)-5′	58.9		
51	5′-d (GTG TGG AAA ATC **F**CT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG **G**GA TCG TCA)-5′	59.0		
52	5′-d (GTG TGG AAA ATC **F**CT AGC AGT)-3′ 3′-d (CAC ACC TTT TAG **T**GA TCG TCA)-5′	57.8		
53	5′-d (GTG TGG AAA ATC TC**F** AGC AGT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	57.2	9.5	13
54	5′-d (GTG TGG AAA ATC TCT AGC **F**GT)-3′ 3′-d (CAC ACC TTT TAG AGA TCG TCA)-5′	59.3	7.5	8.5

[a]) Measured at 260 nm in Na_2HPO_4/NaH_2PO_4 buffer, pH 7, $[Na^+] = 0.15$.

Experimental Part

General. Products were dried under high vacuum. All solvents used were of anh. grade. TLC: precoated silica gel (SiO_2) thin-layer sheets *60 F254* (*Merck*). Flash chromatography (FC): SiO_2 (30–60 μm; *Baker*); 0.2–0.3 bar. Column chromatography (CC): SiO_2 *60*, *Merck*. HPLC: pump *L 6200*, autosampler *AS 4000*, UV detector *L 4000* (*Merck-Hitachi*); column *RP-18*, *Lichrospher 100* (125 × 4 mm, 5 μm; *Merck*); elution: $A = 0.1$M AcO(NHEt$_3$); $B = A +$ MeCN 1:1; A/B 95:5 (0–2 min), A/B 60:40 (30 min), and B (10 min); flow rate 1 ml/min; detection wavelength 260 nm. M.p.: DNA Synthesizer from *Applied Biosystems*, model *392*. UV/VIS: *Perkin-Elmer Lambda 5*; λ_{max} in nm (log ε). ^{1}H-NMR: *Bruker AC 250*; δ in ppm rel. to Me_4Si or $CDCl_3$ ((D$_6$)DMSO) as internal standard, J in Hz. ^{31}P-NMR: *JEOL JMN-GX400*. EI-MS: *Finnigan MAT 8200*; in *m/z* (rel. %). Microanalyses: *Vario Macro Cube, Elementar Analysensysteme GmbH*.

1. *6-[(6-Hydroxyhexyl)amino]uracil* (=*6-[(6-Hydroxyhexyl)amino]pyrimidine-2,4-(1*H,*3*H*)-dione*; **2**). A mixture of 6-chlorouracil (**1**) [16] (2.1 g, 14.3 mmol) and 6-aminohexan-1-ol (3.5 g, 30 mmol) was stirred at 160° for 10 min. After cooling, the precipitate was recrystallized from 80% EtOH in presence of a few drops of AcOH: 2.67 g (82%) of **2**. Colorless crystals. M.p. 208–209°. UV (MeOH): 203 (4.07), 264 (4.37). ^{1}H-NMR ((D$_6$)DMSO): 10.14 (*s*, H–N(1)); 9.80 (*s*, H–N(3)); 6.04 (*t*, HN–C(6)); 4.37 (*s*, H–C(5)); 4.34 (*t*, OH); (*m*, C*H*$_2$OH); 3.37 (*m*, C*H*$_2$NH); 1.40–1.28 (*m*, (C*H*$_2$)$_4$). Anal. calc. for C$_{10}$H$_{17}$N$_3$O$_3$ (227.3): C 52.85, H 7.53, N 18.48; found: C 52.63, H 7.57, N 28.21.

2. *8-Chloro-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**3**). A mixture of **2** (1.1 g, 5 mmol) and 1-chloro-4-nitrosobenzene (1.4 g, 10 mmol) in DMF (20 ml) was stirred at 150° for 30 min and then evaporated. The residue was treated with EtOH, and after cooling, the precipitate was collected: 1.09 g (64%) of **3**. Yellowish crystalline powder. M.p. 255–257° (dec.). UV (MeOH): 223 (4.54), 267 (4.50), 335 (3.86), 430 (4.07). ^{1}H-NMR ((D$_6$)DMSO): 11.43 (*s*, H–N(3)); 8.12 (*d*, H–C(7)); 8.09 (*s*, H–C(9)); 7.66 (*d*, H–C(6)); 4.53 (*m*, CH$_2$N); 4.35 (*t*, OH); 3.38 (*m*, C*H*$_2$OH); 1.67 (*m*, *CH*$_2$CH$_2$N); 1.42 (*m*, (CH$_2$)$_3$). Anal. calc. for C$_{16}$H$_{17}$ClN$_4$O$_3$ (348.8): C 55.09, H 4.91, N 16.06; found: C 54.96, H 4.84, N 15.85.

3. *8-(Dimethylamino)-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**4**). A mixture of **2** (0.23 g, 1 mmol) and *N*,*N*-dimethyl-4-nitrosobenzenamine (0.6 g, 4 mmol) in DMF (10 ml) was stirred at 160° for 3 h and, after cooling, concentrated. The residue was dissolved in CH_2Cl_2 and precipitated by hexane. The dark powder was purified by CC (SiO_2 (3.5 × 12 cm); AcOEt, AcOEt/MeOH 9:1, then $CHCl_3$ and $CHCl_3$/MeOH 9:1): 0.3 g (83%) of **4**. Yellow crystalline powder. M.p. 280–283°. UV (MeOH): 257 (4.72), 312 (3.90), 492 (4.66). ^{1}H-NMR ((D$_6$)DMSO): 10.99 (*s*, H–N(3)); 7.82 (*d*, H–C(6)); 7.22 (*d*, H–C(7)); 6.51 (*s*, H–C(9)); 4.54 (*m*, CH$_2$N); 4.37 (*t*, OH); 3.36 (*m*, C*H*$_2$OH); 3.24 (*s*, 2 Me); 1.70 (*m*, C*H*$_2$CH$_2$N); 1.40 (*m*, (CH$_2$)$_3$). Anal. calc. for C$_{18}$H$_{23}$N$_5$O$_3$ · 0.5 H$_2$O (366.4): C 59.00, H 6.60, N 19.11; found: C 58.92, H 6.51, N 18.83.

4. *8-(Dipropylamino)-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**5**). A mixture of **2** (50 mg, 0.22 mmol) and 1-chloro-4-nitrosobenzene (0.14 g, 1 mmol) in DMF (2 ml) was stirred at 140° for 2 h. Pr_2NH (0.2 ml) was added, and heating at 140° was continued for 2 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 10 cm); AcOEt/MeOH 20:1 → 4:1). The main fraction was recrystallized from EtOH: 40 mg (44%) **5**. Yellow crystals. M.p. 219–220°. UV (MeOH): 258 (4.70), 314 (3.92), 498 (4.66). ^{1}H-NMR ((D$_6$)DMSO): 10.96 (*s*, H–N(3)); 7.79 (*d*, H–C(6)); 7.23 (*d*, H–C(7)); 6.43 (*s*, H–C(9)); 4.52 (*m*, CH$_2$N); 4.36 (*t*, OH); 3.54 (*m*, N(MeCH$_2$C*H*$_2$)$_2$); 3.37 (*m*, C*H*$_2$OH); 1.65 (*m*, 3 *CH*$_2$CH$_2$N); 1.39 (*m*, (CH$_2$)$_3$); 0.94 (*t*, 2 Me). Anal. calc. for C$_{22}$H$_{31}$N$_5$O$_3$ (413.5): C 63.90, H 7.55, N 16.93; found: C 63.87, H 7.50, N 16.93.

5. *8-(Cyclohexylamino)-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**6**). A mixture of **2** (55 mg, 0.25 mmol) and 1-chloro-4-nitrosobenzene (0.14 g, 1 mmol) in $AcNMe_2$ (DMA; 1 ml) was stirred at 140° for 1 h. Cyclohexylamine (1 ml) was added, and heating at 120° was continued for 1 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 15 cm); AcOEt/MeOH 20:1 → 9:1). The main fraction was recrystallized from EtOH/H_2O 1:1: 50 mg (50%) of **6**. Yellow crystals. M.p. 332–336° (dec.). UV (MeOH): 255 (4.62), 312 (3.83), 487 (4.58). ^{1}H-NMR ((D$_6$)DMSO): 10.93 (*s*, H–N(3)); 7.86 (*d*, HN–C(8)); 7.72 (*d*, H–C(6)); 7.05 (*d*, H–C(7)); 6.48 (*s*, H–C(9)); 4.48 (*t*, CH$_2$N); 4.37 (*t*, OH); 3.63 (*m*, C*H*NH); 3.36 (*m*, C*H*$_2$OH); 1.98–1.22 (*m*, (CH$_2$)$_9$). Anal. calc. for C$_{22}$H$_{29}$N$_5$O$_3$ · 2 H$_2$O (447.5): C 59.04, H 7.43, N 15.64; found: C 59.06, H 6.98, N 15.78.

6. *8-(Diethylamino)-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**7**). A mixture of **3** (50 mg, 0.14 mmol) and Et_2NH (0.3 ml) in DMA (2 ml) was stirred at 100° for 1 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 10 cm); AcOEt, then AcOEt/MeOH 20:1). The main fraction was dried: 33 mg (66%) of **7**. Yellow solid. M.p. 260–262°. UV (MeOH): 216 (4.08), 258 (4.71), 315 (3.92), 497 (4.67). ^{1}H-NMR ((D$_6$)DMSO): 10.96 (*s*, H–N(3)); 7.78 (*d*, H–C(6)); 7.20 (*d*, H–C(7)); 6.45 (*s*, H–C(9)); 4.53 (*t*, CH$_2$N); 4.36 (*t*, OH); 3.63 (*q*, MeC*H*$_2$N); 3.37 (*m*, C*H*$_2$OH); 1.69 (*m*, C*H*$_2$CH$_2$N); 1.39 (*m*, (CH$_2$)$_3$); 1.20 (*t*, 2 *Me*CH$_2$). Anal. calc. for C$_{20}$H$_{27}$N$_5$O$_3$ · 0.5 H$_2$O (394.5): C 60.89, H 7.15, N 17.75; found: C 60.85, H 7.25, N 17.79.

7. *10-(6-Hydroxyhexyl)-8-[(phenylmethyl)amino]benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**8**). A mixture of **3** (0.27 g, 0.77 mmol) and benzylamine (3 ml) was stirred at 120° for 1 h and then evaporated. The residue was purified by CC (SiO_2 (2.5 × 15 cm); $CHCl_3$, then $CHCl_3$/MeOH 50:1 → 9:1). The main

fraction was recrystallized from EtOH/$CHCl_3$ 2:1: 0.11 g (34%) of **8**. Yellow crystals. M.p. 295–300° (dec.). UV (MeOH): 254 (4.70), 308 (3.86), 480 (4.67). ^{1}H-NMR ((D_6)DMSO): 10.95 (*s*, H–N(3)); 8.49 (*t*, HN–C(8)); 7.74 (*d*, H–C(6)); 7.36 (*m*, Ph); 7.10 (*d*, H–C(7)); 6.42 (*s*, H–C(9)); 4.59 (*d*, PhCH_2); 4.48 (*t*, CH_2CH_2N); 4.37 (*t*, OH); 3.37 (*m*, CH_2OH); 1.40–1.27 (*m*, $(CH_2)_4$). Anal. calc. for $C_{23}H_{25}N_5O_3$ (419.5): C 65.85, H 6.00, N 16.69; found: C 65.42, H 6.01, N 16.51.

8. *8-[Bis(phenylmethyl)amino]-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**9**). A mixture of **3** (0.1 g, 0.28 mmol) and dibenzylamine (0.5 g, 2.88 mmol) in DMF (1 ml) was stirred at 120° for 5 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 15 cm); $CHCl_3$, then $CHCl_3$/MeOH 20:1 → 9:1): 50 mg (34%) of **9**. Yellow amorphous solid. UV (MeOH): 258 (4.53), 310 (3.86), 488 (4.41). ^{1}H-NMR ((D_6)DMSO): 11.02 (*s*, H–N(3)); 7.81 (*d*, H–C(6)); 7.33 (*m*, H–C(7), 2 Ph); 6.47 (*s*, H–C(9)); 5.08 (*s*, 2 PhCH_2); 4.37 (*t*, OH); 4.35 (*t*, CH_2CH_2N); 3.36 (*m*, CH_2OH); 1.30–1.13 (*m*, $(CH_2)_4$). EI-MS: 509 ($M^{+\bullet}$).

9. *8-(Dodecylamino)-10-(6-hydroxyhexyl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**10**). A mixture of **3** (0.1 g, 0.28 mmol) and dodecan-1-amine (0.212 g, 1.14 mmol) in DMA (4 ml) was stirred at 150° for 0.5 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 25 cm); $CHCl_3$, then $CHCl_3$/MeOH 20:1). The main fraction was recrystallized from MeOH: 120 mg (84%) of **10**. Yellow crystals. M.p. 313–315° (dec.). UV (MeOH): 255 (4.70), 311 (3.85), 484 (4.66). ^{1}H-NMR ((D_6)DMSO): 10.92 (*s*, H–N(3)); 7.94 (*t*, HN–C(8)); 7.71 (*d*, H–C(6)); 7.02 (*d*, H–C(7)); 6.45 (*s*, H–C(9)); 4.48 (*m*, CH_2N(10)); 4.35 (*t*, OH); 3.37 (*m*, CH_2OH); 3.29 (*m*, CH_2NH); 1.60–1.20 (*m*, 12 CH_2); 0.83 (*t*, Me). Anal. calc. for $C_{28}H_{43}N_5O_3$ (498.7): C 67.57, H 8.70, N 14.07; found: C 67.19, H 8.48, N 14.18.

10. *10-(6-Hydroxyhexyl)-8-(tricyclo[3.3.1.1^{3,7}]dec-1-ylamino)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**11**). A mixture of **3** (0.1 g, 0.28 mmol) and adamantan-1-amine (0.42 g, 2.8 mmol) in DMA (4 ml) was stirred at 160° for 9 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 25 cm); AcOEt/MeOH 20:1, then $CHCl_3$/MeOH 20:1). The main fraction was recrystallized from MeOH: 80 mg (60%) of **11**. Yellow crystals. M.p. >330°. UV (MeOH): 255 (4.70), 311 (3.90), 489 (4.68). ^{1}H-NMR ((D_6)DMSO): 10.95 (*s*, H–N(3)); 7.70 (*d*, H–C(6)); 7.55 (*s*, HN–C(8)); 7.17 (*d*, H–C(7)); 6.64 (*s*, H–C(9)); 4.45 (*m*, CH_2N(10)); 4.37 (*t*, OH); 3.37 (*m*, CH_2OH); 2.13–1.40 (*m*, 3 CH, 10 CH_2). EI-MS: 463 ($M^{+\bullet}$). Anal. calc. for $C_{26}H_{33}N_5O_3$ (463.6): C 67.36, H 7.17, N 15.08; found: C 66.90, H 7.17, N 15.08.

11. *10-(6-Hydroxyhexyl)-8-(piperidin-1-yl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**12**). A mixture of **2** (0.1 g, 0.44 mmol) and 1-chloro-4-nitrosobenzene (0.28 g, 2 mmol) in DMF (4 ml) was stirred at 140° for 2 h. Piperidine (4 ml) was added and heating continued for 1 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 20 cm); $CHCl_3$ and $CHCl_3$/MeOH 20:1). The main fraction was recrystallized from EtOH: 104 mg (60%) of **12**. Yellow crystals. M.p. 263–265° (dec.). UV (MeOH): 259 (4.64), 316 (3.83), 497 (4.58). ^{1}H-NMR ((D_6)DMSO): 11.00 (*s*, H–N(3)); 7.78 (*d*, H–C(6)); 7.41 (*d*, H–C(7)); 6.47 (*s*, H–C(9)); 4.54 (*t*, CH_2N); 4.37 (*t*, OH); 3.68 (*s*, 2 CH_2N–C(8)); 3.37 (*m*, CH_2OH); 1.66–1.39 (*m*, 7 CH_2). EI-MS: 397 ($M^{+\bullet}$). Anal. calc. for $C_{21}H_{27}N_5O_3 \cdot 0.5\ H_2O$ (406.5): C 62.05, H 6.94, N 17.22; found: C 62.23, H 6.77, N 17.15.

12. *10-(6-Hydroxyhexyl)-8-(morpholin-4-yl)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**13**). A mixture of **3** (0.14 g, 0.4 mmol) and morpholine (3 ml) in xylene (3 ml) was stirred at 140° for 1 h. After evaporation, the residue was purified by CC (SiO_2 (2.5 × 20 cm); $CHCl_3$ and $CHCl_3$/MeOH 20:1). The main fraction was recrystallized from MeOH/$CHCl_3$ 3:1: 0.12 g (75%) of **13**. Yellow crystals. M.p. 277–279° (dec.). UV (MeOH): 257 (4.56), 310 (3.85), 484 (4.50). ^{1}H-NMR ((D_6)DMSO): 11.07 (*s*, H–N(3)); 7.86 (*d*, H–C(6)); 7.40 (*d*, H–C(7)); 6.83 (*s*, H–C(9)); 4.56 (*m*, CH_2N(10)); 4.36 (*t*, OH); 3.78–3.61 (*m*, CH_2OCH_2); 3.37 (*m*, CH_2OH); 1.69–1.39 (*m*, 6 CH_2). EI-MS: 399 ($M^{+\bullet}$). Anal. calc. for $C_{20}H_{25}N_5O_4 \cdot H_2O$ (417.5): C 57.54, H 6.52, N 16.77; found: C 57.80, H 6.53, N 16.33.

13. *6-{[3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}amino}uracil* (=*6-{[3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}amino}pyrimidine-2,4(1*H,*3*H*)-dione*; **15**). A mixture of 3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propan-1-amine (0.75 g, 4 mmol) and 6-chlorouracil (**1**) [16] (0.3 g, 2 mmol) was stirred at 120° for 20 min. After cooling, the mixture was treated with EtOH and the solid collected, washed with Et_2O, and dried: 0.4 g (65%) of **15**. M.p. 215–216°. UV (MeOH): 264 (4.36). ^{1}H-NMR ((D_6)DMSO): 10.15 (*s*, H–N(1)); 9.91 (*s*, H–N(3)); 6.06 (*t*, CH_2NH); 4.38 (*s*, H–C(5)); 4.16 (*m*, CH); 3.97 (*dd*, CH); 3.59 (*dd*, CH); 3.45 (*t*, CH_2); 3.38 (*dd*, CH_2); 3.04 (*m*,

CH_2CH_2N); 1.69 (*m*, CH_2CH_2N); 1.30, 1.25 (2*s*, 2 Me). EI-MS: 299 ($M^{+\cdot}$). Anal. calc. for $C_{13}H_{21}N_3O_5$ (299.3): C 52.16, H 7.07, N 14.04; found: C 52.04, H 6.99, N 14.14.

14. *6-{{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}amino}-3-methyluracil* (=*6-{{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}amino}-3-methylpyrimidine-2,4(1*H,*3*H*)-dione*; **16**). A mixture of 3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propan-1-amine (1.9 g, 10 mmol) and 6-chloro-3-methyluracil (**14**) [17] (0.8 g, 5 mmol) in *N*-ethyldiisopropylamine (3 ml) was stirred at 120° for 2.5 h. After cooling, the mixture was dissolved in $CHCl_3$ (100 ml) and washed with H_2O (2 × 20 ml), the org. phase dried (Na_2SO_4) and concentrated, and the residue treated with Et_2O and dried: 1.2 g (77%) of **16**. Colorless amorphous powder. UV (MeOH): 264 (4.33). ^{1}H-NMR ((D_6)DMSO): 10.12 (*s*, H–N(1)); 6.08 (*t*, CH_2NH); 4.56 (*s*, H–C(5)); 4.16 (*m*, CH); 3.96 (*dd*, CH); 3.59 (*dd*, CH); 3.40 (*m*, 2 CH_2O); 3.32 (*s*, Me–N(3)): 3.05 (*m*, CH_2CH_2N); 1.70 (*m*, CH_2CH_2N); 1.29, 1.25 (2*s*, 2 Me). EI-MS: 313 ($M^{+\cdot}$). Anal. calc. for $C_{14}H_{23}N_3O_5$ (313.5): C 53.66, H 7.40, N 13.41; found: C 53.64, H 7.25, N 13.13.

15. *8-Chloro-10-{3-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**17**). To a soln. of **15** (2.42 g, 8.08 mmol) in DMA (40 ml), 1-chloro-4-nitrosobenzene (2.28 g, 16.2 mmol) was added gradually, and the mixture was stirred at 110–120° in an oil bath for 5 h. After evaporation, the residue was purified by CC (SiO_2 (5 × 15 cm); $CHCl_3$/MeOH 50:1 → 9:1). The main fraction was recrystallized from $CHCl_3$/MeOH 20:1: 1.6 g (48%) of **17**. Yellow crystals. M.p. 271°. UV (MeOH): 262 (4.22), 313 (3.64), 428 (3.73). ^{1}H-NMR ((D_6)DMSO): 11.43 (*s*, H–N(3)); 8.13 (*d*, H–C(6)); 8.05 (*s*, H–C(9)); 7.67 (*d*, H–C(7)); 4.59 (*t*, CH_2N(10)); 4.17 (*m*, CH); 3.98 (*dd*, CH); 3.59 (*m*, CH, CH_2O); 3.41 (*d*, CH_2O); 2.00 (*m*, CH_2CH_2N); 1.29, 1.25 (2*s*, 2 Me). EI-MS: 420 ($M^{+\cdot}$). Anal. calc. for $C_{19}H_{21}ClN_4O_5$ (420.8): C 52.44, H 5.03, N 13.31; found: C 52.42, H 5.29, N 13.34.

16. *10-{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}-8-[(phenylmethyl)amino]benzo[*g*]-pteridine-2,4(1*H,*3*H*)-dione* (**19**). A mixture of **17** (1.4 g, 3.32 mmol) and benzylamine (3 ml) in DMA (30 ml) was stirred at 120–130° for 30 min and then evaporated. The solid was purified by CC (SiO_2 (5 × 15 cm); $CHCl_3$, then $CHCl_3$/MeOH 40:1). The main fraction was recrystallized from EtOH: 0.6 g (37%) of **19**. Yellow crystals. M.p. 297–298°. UV (MeOH): 254 (4.74), 308 (3.88), 481 (4.71). ^{1}H-NMR ((D_6)DMSO): 10.96 (*s*, H–N(3)); 8.45 (*t*, HN–C(8)); 7.73 (*d*, H–C(6)); 7.41 (*m*, Ph); 7.09 (*d*, H–C(7)); 6.50 (br. *s*, H–C(9)); 4.56 (*d*, CH_2NH); 4.48 (*m*, CH_2N(10)); 4.13 (*m*, CH); 3.99 (*dd*, CH); 3.50 (*m*, CH, CH_2O); 3.38 (*d*, CH_2O); 1.77 (*m*, CH_2CH_2N); 1.27, 1.21 (2*s*, 2 Me). EI-MS: 491 ($M^{+\cdot}$). Anal. calc. for $C_{26}H_{29}N_5O_5$ (491.5): C 63.33, H 5.94, N 14.21; found: C 63.04, H 6.02, N 14.22.

17. *10-{3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]propyl}-8-(dodecylamino)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**20**). As described for **19**, with **17** (0.5 g, 1.2 mmol), dodecan-1-amine (0.55 g, 3 mmol), and DMA (8 ml) at 120° for 3 h. The main CC fraction gave 0.27 g (40%) of **20**. Yellow amorphous powder. UV (MeOH): 255 (4.68), 309 (3.85), 484 (4.58). ^{1}H-NMR ((D_6)DMSO): 10.93 (*s*, H–N(3)); 7.95 (br. *s*, HN–C(8)); 7.71 (*d*, H–C(6)); 7.03 (*d*, H–C(7)); 6.49 (br. *s*, H–C(9)); 4.56 (*t*, CH_2N(10)); 4.15 (*m*, CH); 3.95 (*dd*, CH); 3.58 (*m*, CH, CH_2O); 3.41 (*d*, CH_2O); 1.96 (*m*, CH_2CH_2N); 1.62 (*m*, CH_2CH_2N); 1.29, 1.21 (2*s*, 2 Me); 1.24 (*m*, $(CH_2)_9$); 0.83 (*t*, *Me*CH_2). EI-MS: 569 ($M^{+\cdot}$). Anal. calc. for $C_{31}H_{47}N_5O_5$ (569.7): C 65.35, H 8.31, N 12.29; found: C 65.05, H 8.20, N 12.40.

18. *10-[3-(2,3-Dihydroxypropoxy)propyl]-8-[(phenylmethyl)amino]benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**21**). A soln. of **19** (0.8 g, 1.62 mmol) in 80% AcOH (50 ml) was stirred at 60° for 30 min and then concentrated. EtOH (4 × 30 ml) was added to the residue and co-evaporated; then the residue was dissolved in CH_2Cl_2/MeOH 9:1, the soln. dropwise added into Et_2O (100 ml), and the precipitate recrystallized from EtOH: 0.45 g (61%) of **21**. Yellow crystals. M.p. 276–278°. UV (MeOH): 254 (4.64), 308 (3.80), 481 (4.59). ^{1}H-NMR ((D_6)DMSO): 10.97 (*s*, H–N(3)); 8.46 (*t*, HN–C(8)); 7.73 (*d*, H–C(6)); 7.41 (*m*, Ph); 7.08 (*d*, H–C(7)); 6.55 (br. *s*, H–C(9)); 4.76 (br. *s*, CH_2OH); 4.55 (*d*, CHO*H*, CH_2CH_2NH); 3.60 (*m*, C*H*OH); 3.47 (*m*, 3 CH_2O); 1.77 (*m*, CH_2CH_2N). EI-MS: 451 ($M^{+\cdot}$). Anal. calc. for $C_{23}H_{25}N_5O_5$ (451.5): C 61.19, H 5.58, N 15.51; found: C 60.80, H 5.69, N 15.56.

19. *10-[3-(2,3-Dihydroxypropoxy)propyl]-8-(dodecylamino)benzo[*g*]pteridine-2,4(1*H,*3*H*)-dione* (**22**). A mixture of **17** (0.53 g, 1.33 mmol) and docecan-1-amine (0.555 g, 3 mmol) in DMA (8 ml) was heated to 110° for 4 h and then concentrated. The residue was dissolved in $CHCl_3$ (50 ml), the soln. washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and concentrated, the resulting solid treated in 80% AcOH (50 ml) at 60° for 30 min, and the mixture again concentrated. H_2O (3 × 10 ml) and EtOH (3 × 10 ml) were added to the residue and co-evaporated. The solid was purified by CC (SiO_2 (5 × 12 cm);

$CHCl_3$ and $CHCl_3$/MeOH 40:1 → 9:1). The main fraction was recrystallized from MeOH/$CHCl_3$ 3:1: 0.55 g (78%) of **22**. Yellow crystals. M.p. 255–258°. UV (MeOH): 255 (4.73), 311 (3.92), 485 (4.68). ^{1}H-NMR ((D_6)DMSO): 10.94 (*s*, H–N(3)); 7.94 (*t*, HN–C(8)); 7.72 (*d*, H–C(6)); 7.04 (*d*, H–C(7)); 6.54 (br. *s*, H–C(9)); 4.74 (br. *s*, CH_2OH); 4.54 (*m*, CHO*H*, CH_2CH_2NH); 3.56–3.46 (2*m*, C*H*OH, 3 CH_2O); 1.95 (*m*, CH_2CH_2N); 1.61 (*m*, CH_2CH_2N); 1.21 (*m*, $(CH_2)_9$); 0.82 (*t*, *Me*CH_2). EI-MS: 529 ($M^{+\bullet}$). Anal. calc. for $C_{28}H_{43}N_5O_5$ (529.7): C 63.49, H 8.18, N 13.22; found: C 63.27, H 8.04, N 13.12.

20. *10-[3-(2,3-Dihydroxypropoxy)propyl]-3-methyl-8-[(phenylmethyl)amino]benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**26**). A mixture of **16** (1.0 g, 3.2 mmol) and 1-chloro-4-nitrosobenzene (0.9 g, 6.4 mmol) in DMA (10 ml) was stirred at 120° for 2 h and then concentrated. The residue was dissolved in $CHCl_3$, and the soln. dropwise added into hexane (100 ml). The amorphous precipitate of **18** was collected and dissolved in DMA (10 ml), benzylamine (2 ml) was added and the mixture heated to 120° for 2 h. Evaporation and treatment with hexane gave crude **23** which was dissolved again in 80% AcOH (70 ml). The soln. was stirred at 60° for 1 h and concentrated, H_2O (3 × 10 ml) and EtOH (3 × 20 ml) were added to the residue and co-evaporated. The resulting solid was purified by CC (SiO_2 (5 × 12 cm); $CHCl_3$ and $CHCl_3$/MeOH 40:1 → 20:1). The main fraction was recrystallized from MeOH: 0.5 g (34%) of **26**. Yellow crystals. M.p. 173–176°. UV (MeOH): 254 (4.76), 309 (3.88), 482 (4.71). ^{1}H-NMR ((D_6)DMSO): 8.50 (*t*, HN–C(8)); 7.78 (*d*, H–C(6)); 7.39 (*m*, Ph); 7.10 (*d*, H–C(7)); 6.54 (*s*, H–C(9)); 4.73 (*d*, CHO*H*); 4.54 (*m*, CH_2OH, CH_2NH, $CH_2N(10)$); 3.60–3.30 (*m*, CH, 3 CH_2O); 3.21 (*s*, MeN); 1.78 (*m*, CH_2CH_2N). EI-MS: 465 ($M^{+\bullet}$). Anal. calc. for $C_{24}H_{27}N_5O_5$ (465.5): C 61.92, H 5.84, N 15.04; found: C 61.87, H 5.82, N 15.16.

21. *10-[3-(2,3-Dihydroxypropoxy)propyl]-8-(dodecylamino)-3-methylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**27**). As described for **26**, with 1-chloro-4-nitrosobenzene (1.9 g, 13.4 mmol), **16** (2.1 g, 6.7 mmol), DMA (10 ml), dodecan-1-amine (2.6 g, 14 mmol), and 80% AcOH (100 ml) (*via* **24**). CC (SiO_2 (5 × 12 cm); $CHCl_3$ and $CHCl_3$/MeOH 20:1) and final recrystallization from MeOH gave 1.72 g (47%) of **27**. Yellow crystals. M.p. 173–175°. UV (MeOH): 255 (4.76), 310 (3.90), 486 (4.69). ^{1}H-NMR ((D_6)DMSO): 7.97 (*m*, HN–C(8)); 7.76 (*d*, H–C(6)); 7.05 (*d*, H–C(7)); 6.55 (*s*, H–C(9)); 4.75 (br. *s*, CHO*H*); 4.54 (*m*, CH_2OH, CH_2CH_2N); 3.60–3.30 (*m*, C*H*OH, 3 CH_2O, CH_2NH); 3.21 (*s*, MeN); 1.95 (*m*, CH_2CH_2N); 1.61 (*m*, CH_2CH_2NH); 1.21 (*m*, $(CH_2)_9$); 0.82 (*t*, *Me*CH_2). EI-MS: 543 ($M^{+\bullet}$). Anal. calc. for $C_{29}H_{45}N_5O_5$ (543.7): C 64.06, H 8.34, N 12.88; found: C 63.96, H 8.25, N 12.92.

22. *10-[3-(2,3-Dihydroxypropoxy)propyl]-3-methyl-8-morpholin-4-yl)benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**28**). As described for **26**, with 1-chloro-4-nitrosobenzene (0.9 g, 6.38 mmol), **16** (1.0 g, 3.2 mmol), DMA (5 ml), morpholine (3 ml), and 80% AcOH (70 ml) (*via* **25**). CC (SiO_2 (3 × 12 cm); $CHCl_3$ and $CHCl_3$/MeOH 4:1) and final recrystallization from EtOH gave 0.5 g (39%) of **28**. Yellow crystals. M.p. 245–249°. UV (MeOH): 257 (4.76), 310 (3.89), 485 (4.57). ^{1}H-NMR ((D_6)DMSO): 7.91 (*d*, H–C(6)); 7.40 (*d*, H–C(7)); 6.91 (*s*, H–C(9)); 4.67 (*d*, CHO*H*); 4.62 (*m*, CH_2CH_2NH); 4.54 (*t*, CH_2OH); 3.77–3.44 (*m*, C*H*OH, 5 CH_2O, 2 CH_2N); 3.23 (*s*, MeN); 1.96 (*m*, CH_2CH_2N). EI-MS: 445 ($M^{+\bullet}$). Anal. calc. for $C_{21}H_{27}N_5O_6$ (543.7): C 64.06, H 8.34, N 12.88; found: C 63.96, H 8.25, N 12.92.

23. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-[(phenylmethyl)amino]benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**29**). To a soln. of **21** (0.8 g, 1.77 mmol) in pyridine (20 ml) was added under stirring at r.t. gradually 4,4′-dimethoxytrityl chloride (0.89 g, 2.6 mmol). After 2 h, MeOH (2 ml) was added and the mixture stirred for 15 min, then diluted with $CHCl_3$ (100 ml), and washed with sat. $NaHCO_3$ soln. (30 ml). The org. layer was dried (Na_2SO_4) and concentrated. Toluene (2 × 20 ml) was added to the residue and co-evaporated. The crude material was purified by CC (SiO_2 (5 × 15 cm); AcOEt/MeOH 40:1 → 9:1 containing 0.1% Et_3N). The crude product was dissolved in little CH_2Cl_2 and the soln. added dropwise to hexane. The solid gave, after drying, 0.4 g (30%) of **29**. Amorphous orange-red powder. UV (MeOH): 236 (4.52), 255 (4.66), 488 (4.58). ^{1}H-NMR ((D_6)DMSO): 10.97 (*s*, H–N(3)); 8.41 (*t*, HN–C(8)); 7.74 (*d*, H–C(6)); 7.70–6.75 (*m*, 18 arom. H); 7.09 (*d*, H–C(7)); 6.40 (br. *s*, H–C(9)); 5.04 (br. *s*, CHO*H*); 4.43 (*d*, CH_2NH); 4.31 (*m*, $CH_2N(10)$); 3.81 (*m*, C*H*OH); 3.63 (*s*, 2 MeO); 3.43 (*m*, 2 CH_2O); 2.96 (*m*, CH_2O); 1.75 (*m*, CH_2CH_2N). Anal. calc. for $C_{44}H_{43}N_5O_7$ (753.8): C 70.10, H 5.75, N 9.29; found: C 69.44, H 5.66, N 9.31.

24. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-(dodecylamino)benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**30**). As described for **29**, with **22** (0.5 g, 0.9 mmol), dimethoxytrityl chloride (0.7 g, 2 mmol), and pyridine (10 ml). CC (SiO_2 (3.5 × 12 cm); $CHCl_3$/MeOH 100:1 → 50:1) gave 0.3 g

(39%) of **30**. Amorphous orange-red powder. UV (MeOH): 236 (4.52), 255 (4.65), 486 (4.60). ^{1}H-NMR ((D$_6$)DMSO): 10.95 (*s*, H–N(3)); 7.90 (*t*, HN–C(8)); 7.72 (*d*, H–C(6)); 7.40–6.74 (*m*, 13 arom. H); 7.03 (*d*, H–C(7)); 6.30 (br. *s*, H–C(9)); 5.08 (br. *s*, CHO*H*); 4.35 (*m*, CH$_2$N(10)); 3.82 (*m*, C*H*OH); 3.63 (*s*, 2 MeO); 3.56–2.99 (*m*, C*H*$_2$NH, 3 CH$_2$O); 1.87 (*m*, C*H*$_2$CH$_2$N); 1.54 (*m*, C*H*$_2$CH$_2$NH); 1.19 (*m*, (CH$_2$)$_7$); 0.82 (*t*, *Me*CH$_2$). Anal. calc. for $C_{49}H_{61}N_5O_7$ (832.1): C 70.73, H 7.39, N 8.42; found: C 70.31, H 7.38, N 8.40.

25. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-3-methyl-8-[(phenylmethyl)-amino]benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**31**). As described for **29**, with **26** (0.824 g, 1.77 mmol), 4,4′-dimethoxytrityl chloride (0.89 g, 2.6 mmol), and pyridine (20 ml). CC (SiO$_2$ (3 × 12 cm); AcOEt/MeOH 100:1 → 50:1) gave 0.66 g (84%) of **31**. Amorphous orange-red powder. UV (MeOH): 237 (4.50), 254 (4.72), 482 (4.66). ^{1}H-NMR ((D$_6$)DMSO): 8.45 (*t*, HN–C(8)); 7.78 (*d*, H–C(6)); 7.40–6.70 (*m*, 19 arom. H, H–C(7)); 6.41 (br. *s*, H–C(9)); 5.02 (br. *s*, CHO*H*); 4.44 (*d*, C*H*$_2$NH); 4.34 (*m*, CH$_2$N(10)); 3.79 (*m*, C*H*OH); 3.63, 3.62 (2*s*, 2 MeO); 3.43–2.96 (*m*, 3 CH$_2$O); 3.21 (*s*, MeN); 1.75 (*m*, C*H*$_2$CH$_2$N). Anal. calc. for $C_{45}H_{45}N_5O_7$ (785.9): C 68.77, H 5.77, N 8.91; found: C 68.12, H 5.99, N 8.31.

26. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-(dodecylamino)-3-methylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**32**). As described for **29**, with **27** (1.14 g, 2.1 mmol), 4,4′-dimethoxytrityl chloride (1.02 g, 3.1 mmol), and pyridine (20 ml). CC (SiO$_2$ (5 × 12 cm); CHCl$_3$/MeOH 100:1 containing 0.1% Et$_3$N) gave 1.2 g (68%) of **32**. Amorphous orange-red solid. UV (MeOH): 237 (4.50), 254 (4.74), 486 (4.67). ^{1}H-NMR ((D$_6$)DMSO): 7.92 (*t*, HN–C(8)); 7.74 (*d*, H–C(6)); 7.40–6.70 (*m*, 13 arom. H); 7.04 (*d*, H–C(7)); 6.32 (br. *s*, H–C(9)); 5.05 (br. *s*, CHO*H*); 4.37 (*m*, CH$_2$N(10)); 3.82 (*m*, C*H*OH); 3.61, 3.60 (2*s*, 2 MeO); 3.55–2.95 (*m*, 3 CH$_2$O, C*H*$_2$NH); 3.21 (*s*, MeN); 1.88 (*m*, C*H*$_2$CH$_2$N); 1.54 (*m*, C*H*$_2$CH$_2$NH); 1.18 (*m*, (CH$_2$)$_8$); 0.81 (*t*, *Me*CH$_2$). Anal. calc. for $C_{50}H_{63}N_5O_7$ (846.1): C 70.98, H 7.50, N 8.28; found: C 70.64, H 7.46, N 8.05.

27. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-3-methyl-8-(morpholin-4-yl)-benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione* (**33**). As described for **29**, with **28** (1.12 g, 2.5 mmol), 4,4′-dimethoxytrityl chloride (1.02 g, 3.1 mmol), and pyridine (20 ml). CC (SiO$_2$ (3 × 12 cm); CHCl$_3$ and CHCl$_3$/MeOH 100:1 containing 0.1% Et$_3$N) gave 0.8 g (36%) of **33**. Amorphous orange-red solid. UV (MeOH): 235 (4.49), 259 (4.59), 487 (4.67). ^{1}H-NMR ((D$_6$)DMSO): 7.90 (*d*, H–C(6)); 7.5–6.75 (*m*, 13 arom. H); 7.39 (*d*, H–C(7)); 6.66 (br. *s*, H–C(9)); 4.97 (br. *s*, CHO*H*); 4.42 (*m*, CH$_2$N(10)); 3.78 (*m*, C*H*OH); 3.61, 3.60 (2*s*, 2 MeO); 3.70–2.97 (*m*, 5 CH$_2$O, C*H*$_2$NC*H*$_2$); 3.23 (*s*, MeN); 1.88 (*m*, CH$_2$C*H*$_2$CH$_2$N). Anal. calc. for $C_{42}H_{45}N_5O_8$ (747.85): C 76.45, H 6.06, N 9.36; found: C 76.03, H 7.15, N 9.12.

28. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-[(phenylmethyl)amino]benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione 2″-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**34**). To a mixture of **29** (0.35 g, 0.46 mmol) and 1*H*-tetrazole (16 mg, 0.23 mmol) in CH$_2$Cl$_2$ (4 ml) was added 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.21 g, 0.69 mmol) at r.t. and stirred under Ar for 8 h. The mixture was diluted with CHCl$_3$ (50 ml) and washed with sat. NaHCO$_3$ soln. (2 × 30 ml), the org. layer dried (Na$_2$SO$_4$) and concentrated, the residue dissolved in CH$_2$Cl$_2$, and the soln. added dropwise into hexane. The amorphous powder was dried *in vacuo:* 0.39 g (67%) of **34**. UV (MeOH): 236 (4.63), 254 (4.77), 482 (4.71). ^{31}P-NMR (CDCl$_3$): 150.074; 149.942; 149.397. Anal. calc. for $C_{53}H_{60}N_7O_8P$ (954.1): C 66.72, H 6.38, N 10.27; found: C 66.26, H 6.32, N 10.15.

29. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-(dodecylamino)benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione 2″-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**35**). As described for **34**, with **30** (0.52 g, 0.62 mmol), 1*H*-tetrazole (21 mg, 0.3 mmol), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.26 g, 0.87 mmol), and CH$_2$Cl$_2$ (7 ml). The amorphous powder was dried *in vacuo:* 0.5 g (77%) of **35**. UV (MeOH): 235 (4.62), 255 (4.72), 486 (4.68). ^{31}P-NMR (CDCl$_3$): 149.736. Anal. calc. for $C_{58}H_{78}N_7O_8P$ (1032.3): C 67.48, H 7.61, N 9.49; found: C 67.25, H 7.65, N 9.20.

30. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-3-methyl-8-[(phenylmethyl)-amino]benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione 2″-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**36**). As described for **34**, with **31** (0.4 g, 0.52 mmol), 1*H*-tetrazole (17 mg, 0.24 mmol), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.22 g, 0.73 mmol), and CH$_2$Cl$_2$ (5 ml). The amorphous powder was dried *in vacuo:* 0.39 g (77%) of **36**. UV (MeOH): 235 (4.64), 254 (4.72), 483 (4.68). ^{31}P-

NMR ($CDCl_3$): 149.915; 149.832; 149.298. Anal. calc. for $C_{54}H_{62}N_7O_8P$ (968.1): C 66.99, H 6.45, N 10.13; found: C 66.72, H 6.52, N 9.87.

31. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-8-(dodecylamino)-3-methylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione 2″-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**37**). As described for **34**, with **32** (1.14 g, 1.34 mmol), 1*H*-tetrazole (47 mg, 0.7 mmol), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.61 g, 2.0 mmol), and CH_2Cl_2 (15 ml). The amorphous powder was dried *in vacuo:* 1.2 g (85%) of **37**. UV (MeOH): 234 (4.65), 255 (4.69), 487 (4.69). ^{31}P-NMR ($CDCl_3$): 149.652. Anal. calc. for $C_{59}H_{80}N_7O_8P$ (1045.7): C 67.76, H 7.71, N 9.37; found: C 67.21, H 7.26, N 9.52.

32. *10-{3-{3-[(4,4′-Dimethoxytrityl)oxy]-2-hydroxypropoxy}propyl}-3-methyl-8-(morpholin-4-yl)-benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione 2″-[2-Cyanoethyl* N,N-*Diisopropylphosphoramidite]* (**38**). As described for **34**, with **33** (0.28 g, 0.37 mmol), 1*H*-tetrazole (13 mg, 0.19 mmol), 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.17 g, 0.56 mmol), and CH_2Cl_2 (5 ml). The amorphous powder was dried *in vacuo:* 0.25 g (70%) of **38**. UV (MeOH): 235 (4.65), 257 (4.64), 487 (4.55). ^{31}P-NMR ($CDCl_3$): 150.250; 149.574. Anal. calc. for $C_{51}H_{62}N_7O_9P$ (948.1): C 64.61, H 6.59, N 10.34; found: C 64.50, H 6.81, N 9.56.

33. *Oligodeoxyribonucleotide Syntheses.* The solid-support material *LCAMA-CPG* 500 Å was prepared according to [12–14], and loading with 5′-*O*-4,4′-(dimethoxytrityl)-3′-*O*-succinylthymidine was achieved in the usual manner [14]. The assembly of the oligodeoxyribonucleotides in an *Applied-Biosystems-392* synthesizer was performed with 2-(4-nitrophenyl)ethyl(npe)/[2-(4-nitrophenyl)ethoxy]-carbonyl(npeoc)-protected 2′-deoxyribonucleoside 3′-(2-cyanoethyl *N,N*-diisopropylphosphoramidites) [15].

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