DOI: 10.1002/ejoc.200700234

The Fluorescence Labelling of Primary Amines with Perylenetetracarboxdiimides

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Keywords: UV/Vis spectroscopy / Fluorescent probes / Amines / Dyes / Pigments / Schiff bases

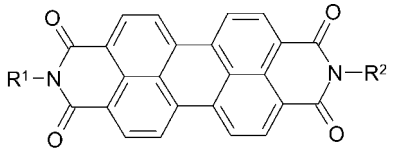
The synthesis of perylenetetracarboxdiimide-labelled aldehydes is described as well as their condensation with primary amines. Thus, a highly fluorescent and light-fast fluorescence labelling process has been demonstrated. Application of this method to biological targets is also reported.

Introduction

Fluorescence labelling of biological targets is becoming more and more important.[1] Many biologically active structures contain primary amino groups which can be labelled by condensation reactions. Such primary amines react easily with aldehydes, particularly aromatic aldehydes, to form aldimines[2,3] in high yields. Fluorophores with aldehyde anchor groups are interesting reagents for such labelling.

Results and Discussion

We started with the highly photostable,[4–7] highly fluorescent[8] and chemically inert[9] perylenetetracarboxdiimides **1**[10,11] as the fluorophore and attached the long-chain *sec*-alkyl substituent 1-hexylheptyl ("swallow-tail" substituent[12,13]) to one of the nitrogen atoms of **1** (R^1) to render the material soluble. A group containing an aromatic aldehyde attached to the other nitrogen atom of **1** (R^2) would be a suitable anchor for labelling.



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We started the synthesis with 4-cyanobenzaldehyde (**2**) and protected the aldehyde group as the dimethyl acetal **3**[14,15] because a free amino group would cause self-condensation. The cyano group was reduced[16] with lithium aluminium hydride to the amine **5**. Condensation of **5** with the *N*-(1-hexylheptyl)perylene anhydride imide[17] was successful, however, the acetal **1a** was very labile and hydrolysed during the usual workup to the aldehyde **1c**. Thus, the methyl acetal is a good starting material for a one-step synthesis of the aldehyde **1c** (Scheme 1).

We prepared the more stable ethylene acetal **4**[16] for long-term storage of the aldehyde, reduced it analogously to the amine **6** and condensed it with *N*-(1-hexylheptyl)perylene anhydride imide to form the acetal **1b**. Standard chromatographic purification of **1b** (silica gel 60; $CHCl_3$/ethanol, 40:1) caused hydrolysis to the aldehyde **1c**, as was found for **1a**. Even chromatographic separation with basic alumina caused partial hydrolysis and the formation of the alcohol **1f**. This may be a consequence of the reduction of **1c** by the ethanol used for elution or alternatively the basic alumina may cause a Cannizzaro reaction of **1c**. However, the corresponding carboxylic acid could not be isolated. On the other hand, chromatographic purification of the raw material with the deactivated silica Florisil allowed the isolation of the acetal **1b**, a material that allows for the long-term storage of **1c**. The aldehyde **1c** can be liberated from **1b** by simple column chromatographic filtration (silica gel 60; $CHCl_3$/ethanol, 40:1).

The labelling group in **1c** is close to the chromophore and there may be interference for some substrates by interaction with the chromophore. Thus, to avoid this, we separated the labelling aldehyde group from the chromophore by an additional phenyl group to provide a longer spacer with low flexibility. Therefore, the bromo nitrile **7** was coupled with the boronic aldehyde **8** in a Suzuki reaction[18] with tetrakis(triphenylphosphane)palladium to form the biphenyl aldehyde **9**. The latter was protected as the ethylene acetal **10**, reduced to the amine **11** and condensed with *N*-(1-hexylheptyl)perylene anhydride imide to form **1d**. The acetal **1d** could be obtained by column chromatography with the deactivated silica Florisil or alternatively with silica gel 60 using dichloromethane/methanol (40:1) as eluent,

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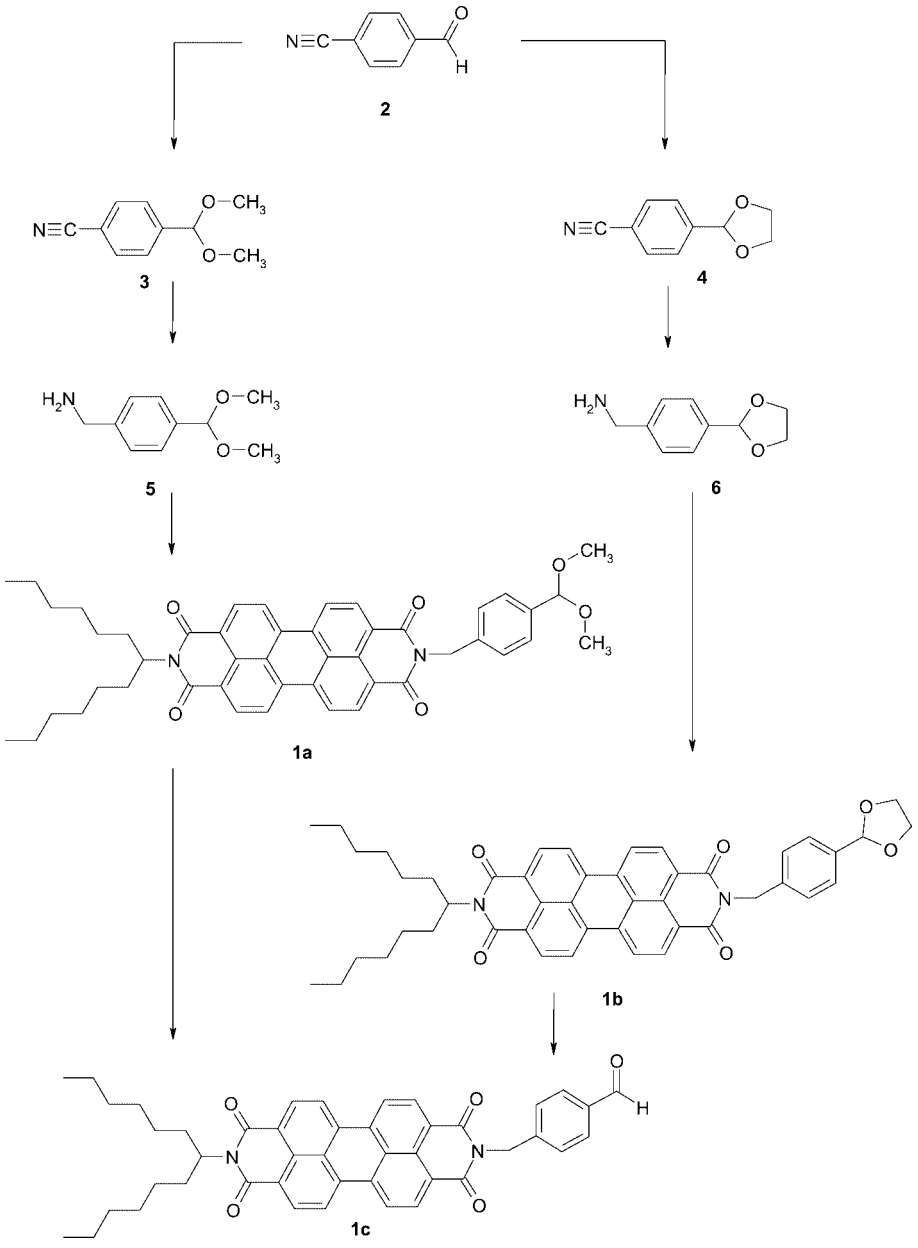
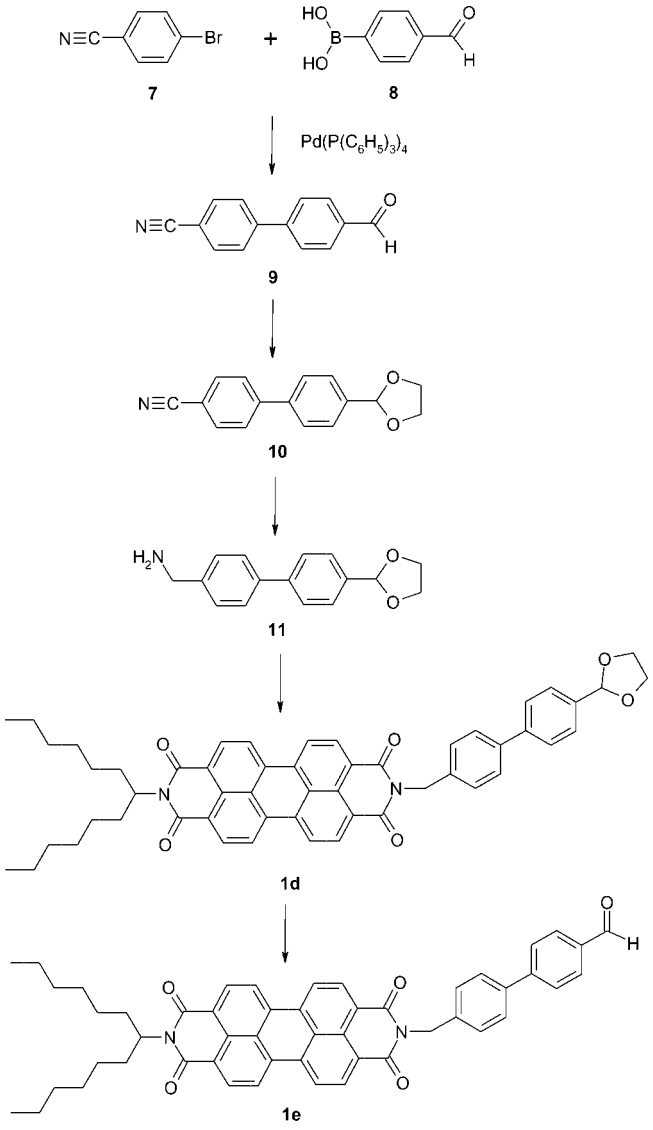


Scheme 1. Synthesis of the perylene dye **1c**.

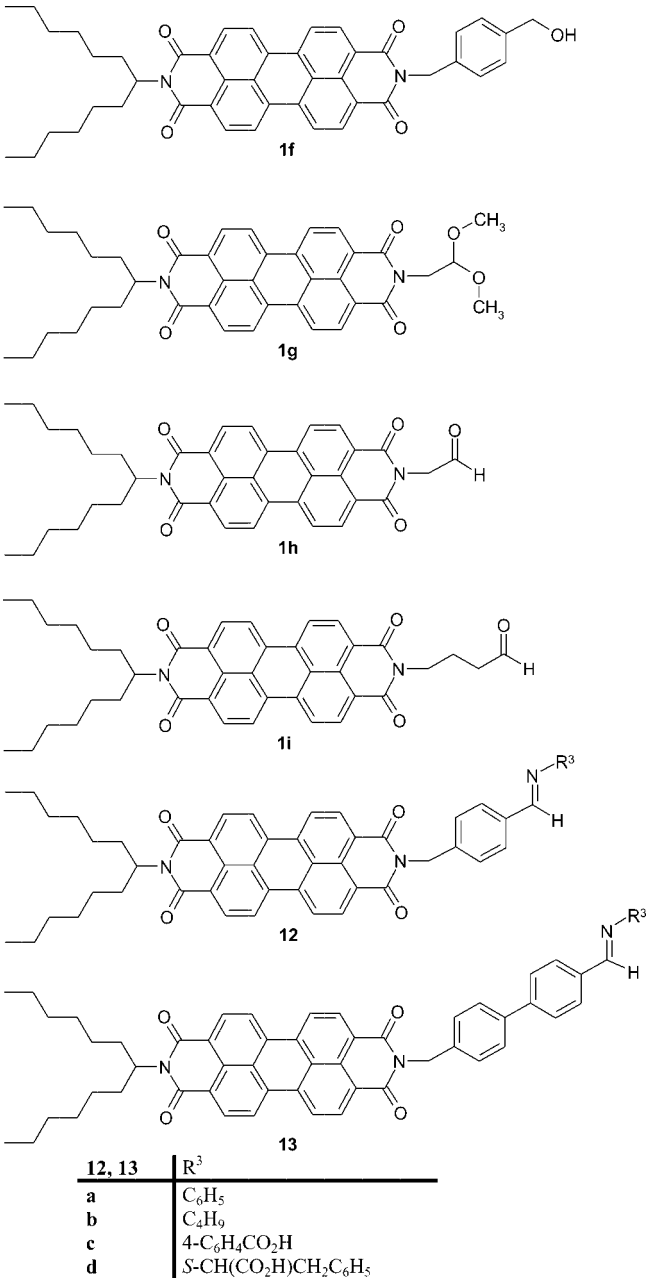
whereas common silica gel 60, $CHCl_3$/ethanol (40:1) allowed deprotection to yield the free aldehyde **1e**, similarly to the behaviour of **1b** (Scheme 2).

Simple aliphatic aldehydes were prepared for comparison. The condensation of the readily accessible perylene-3,4:9,10-tetracarboxylic 3,4-anhydride monopotassium salt with 4-aminobutyraldehyde diethyl acetal was successful with direct hydrolysis of the acetal during workup yielding the corresponding aldehyde anhydride; however, the condensation of this aldehyde with branched aliphatic amines to form compounds such as **1i** proved to be problematic. Thus, a route analogous to that used for the preparation of **1c** was preferred in which the *N*-(1-hexylheptyl)perylene-3,4:9,10-tetracarboxylic 3,4-anhydride 9,10-imide was condensed with 2-aminoacetaldehyde dimethyl acetal and 4-aminobutyraldehyde diethyl acetal to form **1h** and **1i**, respectively. The lower reactivity of the aliphatic acetals was indicated by the fact that the dimethyl acetal **1g** was obtained by standard acidic workup without hydrolysis so that the preparation of the aldehyde by hydrolysis requires an additional step. The ethyl acetal of **1i** proved to be more labile to acid so that hydrolysis proceeded directly to **1i**.

The condensation of **1c** and **1e** with primary aromatic amines was first verified with aniline to form the corresponding aldimines **12a** and **13a**, respectively, in 70% yields. Remarkably, even aliphatic amines such as butylamine reacted readily to form aldimines; see for example **12b** and **13b**, respectively.



Scheme 2. Synthesis of the perylene dye **1e**.



12, 13	R^3
a	C_6H_5
b	C_4H_9
c	4-$C_6H_4CO_2H$
d	*S*-$CH(CO_2H)CH_2C_6H_5$

The aliphatic aldehydes **1h** and **1i** underwent condensation with butylamine to form the highly fluorescent, but readily hydrolysable aldimines in low yields of about 15% with *m*/*z* = 669 and 697, respectively. The condensation of **1h** with aniline proceeded with an even lower yield of 10% (*m*/*z* = 689) and no aldimine could be detected for the condensation of **1i** with aniline by mass spectrometry.

The procedure for labelling had to be modified for more hydrophilic biologically important amines such as amino acids. Ethanol proved to be a sufficiently good solvent for both the amino acids and the perylene-labelled aldehydes. Thus, we condensed 4-aminobenzoic acid as the amino acid with the aldehydes **1c** and **1e** and obtained the imines **12c** and **13c**, respectively. *N*-Methylpyrrolidone proved to give more satisfying results for the even more polar natural α-amino acids; phenylalanine was used as an example to prepare **12d** and **13d**, respectively.

Moreover, the labelling of peptides with **1c** and **1e** is possible, as was shown by the reaction of catalase. *N*-Methylpyrrolidone gave the best results and the labelling could be further supported by the addition of dicyclohexylcarbodiimide (DCC). Orange-to-dark-red peptides were formed, the coloration of which could not be removed by hydrophilic or lipophilic solvents such as chloroform. This indicates a covalent linkage of the dye to the peptide.

All aldimines from **12a** to **13d** exhibit UV/Vis absorption and strong fluorescence spectra very similar to the starting material; see the comparison of the UV/Vis spectra of **12b** and a simple aliphatically substituted reference shown in Figure 1. The photostability of the labelled amines is relatively high, as has been found for other perylene dyes. The

chemical stability of the labelling linkage is, for example, high enough for chromatographic separation. The fluorescence of the labelled peptide is not as pronounced as that of labelled amino acids which may be a consequence of the aggregation of chromophores in the highly loaded macromolecular peptide.

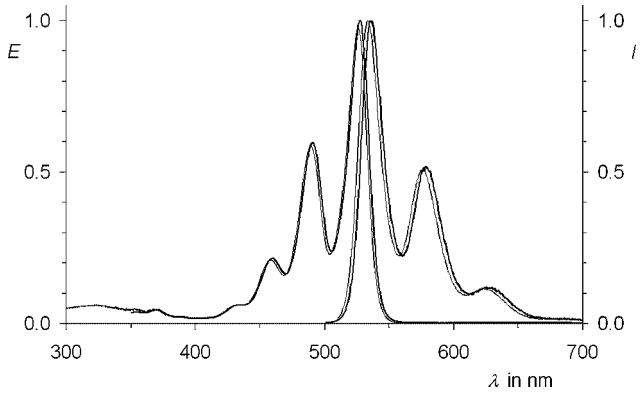
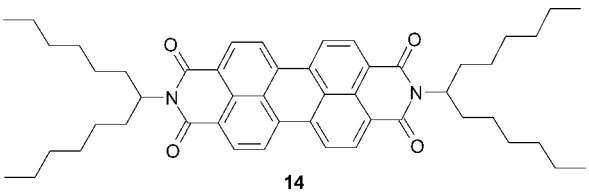


Figure 1. Normalised UV/Vis absorption (left) and fluorescence (right) spectra of **13a** (thick line) and **14** (thin line) in chloroform.



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Conclusions

Aldehydes **1c** and **1e** are useful reagents for labelling both lipophilic primary amines and hydrophilic amino acids and their use can even be extended to the labelling of biological macromolecules.

Experimental Section

General: IR spectra: Perkin Elmer 1420 Ratio Recording Infrared Spektrometer, FT 1000. UV/Vis spectra: Varian Cary 5000 and Bruins Omega 20. Fluorescence spectra: Perkin Elmer FS 3000 (totally corrected). NMR spectra: Varian Vnmrs 600 (600 MHz). Mass spectra: Finnigan MAT 95. Perylene-3,4:9,10-tetracarboxylic bis(anhydride) was obtained from CIBA Speciality Chemicals, other staring materials from Aldrich.

2-[4-(Dimethoxymethyl)benzyl]-9-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (1a) and Splitting of the Acetal To Form 4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]benzaldehyde (1c): 9-(1-Hexylheptyl)-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]isoquinoline-1,3,8,10-tetraone (860 mg, 1.50 mmol), imidazole (17.0 g) and a microspatulum of zinc acetate [$Zn(OAc)_2 \cdot 2H_2O$] were homogenized, heated under argon at 140 °C, treated with **5** (460 mg, 2.55 mmol), heated at 140 °C for 2 h with stirring, cooled, treated with ethanol (50 mL), precipitated with aqueous 2 M HCl (150 mL), collected by vacuum filtration, thoroughly washed with distilled water and dried in air at 110 °C for 16 h to obtain **1a**. The splitting to form **1c** proceeded by purification by column chromatography (silica gel; chloroform/ethanol, 40:1). Compound **1c** was obtained after an orange forerun as an intense red-to-orange band and was dissolved in a small amount of chloroform and precipitated with acetonitrile. Yield: 785 mg (76%). R_f (silica gel; $CHCl_3$/EtOH, 40:1) = 0.29. IR (ATR): $\tilde{\nu}$ = 2953.8 (m), 2923.0 (s), 2855.3 (m), 1697.4 (s), 1646.4 (vs), 1610.0 (m), 1593.4 (s), 1577.3 (m), 1506.9 (w), 1436.2 (m), 1403.9 (m), 1378.2 (w), 1336.1 (s), 1301.7 (w), 1249.8 (m), 1212.4 (w), 1199.7 (w), 1168.2 (m), 1125.2 (w), 1106.0 (w), 987.0 (w), 849.6 (w), 823.9 (w), 808.9 (m), 774.3 (w), 742.6 (m), 723.1 (w), 631.4 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.82 [t, ^3J(H,H) = 6.9 Hz, 6 H, 2 × CH_3], 1.16–1.38 (m, 16 H, 8 × CH_2), 1.82–1.92 (m, 2 H, β-CH_2), 2.20–2.30 (m, 2 H, β-CH_2), 5.14–5.22 (m, 1 H, α-CH), 5.48 (s, 2 H, NCH_2), 7.70 (d, ^3J = 8.2 Hz, 2 H, CH_{aryl}), 7.85 (d, ^3J = 8.3 Hz, 2 H, CH_{aryl}), 8.68 (m, 8 H, CH_{aryl}), 9.98 (s, 1 H, CHO) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 14.3, 22.8, 27.1, 29.4, 29.9, 32.0, 32.6, 43.8, 55.1, 123.0, 123.2, 123.6, 126.6, 129.6, 129.8, 130.2, 132.1, 135.2, 135.9, 144.0, 163.6, 192.1 ppm. UV/Vis ($CHCl_3$): λ_{max} (ε) = 459.1 (18600), 491.0 (51400), 527.4 (85800) nm; fluorescence ($CHCl_3$): λ_{max} (I_{rel}) = 534.5 (1.00), 578.0 (0.50) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 491 nm, E_{491nm} = 0.0212 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 690 (33) $[M]^+$, 508 (100) $[M - C_{13}H_{26}]^+$, 374 (14) $[M - C_{21}H_{35}O_2]^+$, 346 (19) $[M - C_{22}H_{34}NO_2]^+$, 44 (15) $[CH_2NO]^+$. HMRS: calcd. for $C_{45}H_{42}N_2O_5$ $[M]^+$ 690.309; found 690.308. $C_{45}H_{42}N_2O_5$ (690.9): calcd. C 78.24, H 6.13, N 4.06; found C 78.06, H 6.15, N 4.07.

2-[4-(1,3-Dioxolan-2-yl)benzyl]-9-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (1b) and Hydrolysis To Form 4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]benzaldehyde (1c) and the Isolation of 2-(1-Hexylheptyl)-9-(4-hydroxymethylbenzyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (1f): 9-(1-Hexylheptyl)-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]isoquinoline-1,3,8,10-tetraone (600 mg, 1.05 mmol), imidazole (14.0 g) and a microspatulum of zinc acetate [$Zn(OAc)_2 \cdot 2H_2O$] were homogenized and heated under argon (deep-red solution at 140 °C), treated with **6** (370 mg, 2.06 mmol), stirred for 4 h, quenched by the addition of ethanol (50 mL), precipitated with 2 N aqueous HCl, cooled, collected by vacuum filtration, washed thoroughly with distilled water, dried in air (110 °C, 16 h, 740 mg, 96%), purified by column chromatography (Florisil; chloroform/ethanol, 60:1), dissolved in chloroform and precipitated with acetonitrile. Yield: 12 mg of **1b** (16%) (alternatively 60% yield of **1b** with silica gel 60; dichloromethane/methanol, 40:1). M.p. >250 °C. R_f (silica gel; CH_2Cl_2) = 0.23. IR (ATR): $\tilde{\nu}$ = 2954.5 (m), 2921.7 (s), 2854.0 (s), 2360.6 (w), 1683.2 (s), 1648.1 (vs), 1592.5 (s), 1575.8 (m), 1506.3 (w), 1465.9 (w), 1436.4 (w), 1402.4 (m), 1338.8 (s), 1306.4 (w), 1249.5 (m), 1203.6 (w), 1173.8 (m), 1126.3 (w), 1109.8 (w), 1083.9 (m), 1019.9 (w), 981.7 (w), 944.6 (w), 852.0 (w), 809.9 (s), 780.0 (w), 744.7 (m), 666.7 (w), 647.5 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.82 [t, ^3J(H,H) = 6.9 Hz, 6 H, 2 × CH_3], 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2 × CH_3], 1.18–1.38 (m, 16 H, 8 × CH_2), 1.54 (s, 1 H, OH), 1.83–1.91 (m, 2 H, β-CH_2), 2.21–2.29 (m, 2 H, β-CH_2), 4.66 [d, ^3J(H,H) = 3.9 Hz, 2 H, CH_2OH], 5.15–5.22 (m, 1 H, α-CH), 5.40 (s, 2 H, NCH_2), 7.34 [d, ^3J(H,H) = 8.1 Hz, 2 H, CH_{aryl}], 7.58 [d, ^3J(H,H) = 8.2 Hz, 2 H, CH_{aryl}], 8.57–8.72 (m, 8 H, CH_{aryl}) ppm. ^{13}C NMR (150 MHz,

$CDCl_3$, 25 °C): δ = 14.3, 22.8, 27.1, 29.4, 32.0, 32.6, 43.7, 55.0, 65.5, 103.7, 123.2, 123.3, 123.4, 126.6, 126.8, 129.4, 129.7, 129.8, 131.9, 135.2, 137.6, 138.2, 163.6 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.4 (0.22), 490.4 (0.60), 527.2 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 534.5 (1.00), 576.0 (0.51) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0137 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. HMRS: calcd. for $C_{47}H_{46}N_2O_6$ $[M]^+$ 734.336; found 734.337. Column separation with silica gel (chloroform/ethanol, 40:1) results directly in the splitting of the acetal to form **1c** as an intense reddish-orange band after having removed a forerun and was dissolved in chloroform and precipitated with acetonitrile. Yield: 470 mg (74%) of **1c**; for spectroscopic data see below. Column separation of the crude acetal with neutral alumina (chloroform/ethanol, 40:1) gave a weakly orange forerun and the main fraction. Further column chromatography of the latter with silica gel (chloroform/ethanol, 60:1) allowed pure aldehyde **1c** to be obtained and then **1f**. Yield: 25 mg. Red powder, m.p. >250 °C. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.15. IR (ATR): $\tilde{\nu}$ = 3500.0 (br, w), 2953.8 (w), 2923.6 (m), 2855.6 (w), 2360.4 (m), 2340.5 (m), 1693.4 (s), 1649.9 (vs), 1593.8 (s), 1576.1 (m), 1507.2 (w), 1437.4 (w), 1403.8 (m), 1344.3 (s), 1250.2 (m), 1173.3 (m), 1128.9 (w), 1018.0 (m), 852.2 (w), 824.5 (w), 809.9 (s), 784.1 (w), 753.1 (m), 667.9 (w), 645.9 (w), 629.4 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.82 (t, ^3J = 6.5 Hz, 6 H, 2 × CH_3), 1.28 (m, 16 H, CH_2), 1.57 (s, 1 H, OH), 1.87 (m, 2 H, α-CH_2), 2.23 (m, 2 H, α-CH_2), 4.66 (s, 2 H, CH_2OH), 5.18 (m, 1 H, α-CH), 5.40 (s, 2 H, NCH_2), 7.34 (d, ^3J = 8.1 Hz, 2 H, CH_{aryl}), 7.58 (d, ^3J = 8.1 Hz, 2 H, CH_{aryl}), 8.68 (m, 8 H, CH_{aryl}) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 14.3, 22.8, 27.2, 29.4, 32.0, 32.6, 43.7, 55.0, 65.4, 123.2, 123.3, 123.4, 126.6, 127.4, 129.6, 129.7, 131.9, 135.2, 136.8, 140.5, 163.6 ppm. HMRS: calcd. for $C_{45}H_{44}N_2O_5$ $[M]^+$ 692.325; found 692.323.

2-[4′-(1,3-Dioxolan-2-yl)biphenyl-4-ylmethyl]-9-(1-hexylheptyl)-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (1d): 9-(1-Hexylheptyl)-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]-isoquinoline-1,3,8,10-tetraone (400 mg, 0.697 mmol), **11** (350 mg, 1.37 mmol), imidazole (10.0 g) and a microspatulum of zinc acetate [$Zn(OAc)_2 \cdot 2H_2O$] under argon were allowed to react as was described for **1a**. Yield: 550 mg (0.599 mmol, 86%). Bright light-red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.27. IR (ATR): $\tilde{\nu}$ = 2956.4 (m), 2923.8 (s), 2855.8 (m), 1692.2 (s), 1653.5 (vs), 1594.0 (s), 1578.2 (m), 1505.3 (w), 1457.1 (w), 1434.7 (w), 1403.9 (w), 1378.4 (w), 1351.8 (m), 1330.5 (s), 1246.7 (m), 1215.6 (w), 1170.5 (w), 1124.5 (w), 1107.6 (w), 1071.2 (w), 1026.0 (w), 1006.3 (w), 977.1 (w), 855.5 (w), 809.7 (m), 743.5 (m), 633.6 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2 × CH_3], 1.18–1.38 (m, 16 H, 8 × CH_2), 1.83–1.91 (m, 2 H, β-CH_2), 2.21–2.29 (m, 2 H, β-CH_2), 4.02–4.16 (m, 4 H, 2 × CH_2O), 5.15–5.22 (m, 1 H, α-CH), 5.45 (s, 2 H, NCH_2), 5.84 (s, 1 H, CHO_2), 7.49–7.73 (m, 8 H, CH_{aryl}), 8.58–8.72 (m, 8 H, $CH_{perylene}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.3, 22.8, 27.2, 29.4, 32.0, 32.6, 43.7, 55.1, 63.9, 123.2, 123.5, 126.6, 126.8, 127.1, 127.3, 127.5, 129.8, 129.9, 130.5, 132.0, 135.3, 135.4, 137.7, 139.3, 147.0, 163.7 ppm. UV/Vis ($CHCl_3$): λ_{max} (E_{rel}) = 459.6 (0.22), 490.8 (0.60), 527.6 (1.00); fluorescence ($CHCl_3$): λ_{max} (I_{rel}) = 535.5 (1.00), 578.5 (0.52), 628.5 nm (0.12); fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0302 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI^+, 70 eV): *m/z* (%) = 810 (45) $[M]^+$, 628 (62) $[M - C_{13}H_{26}]^+$, 585 (78) $[M - C_{15}H_{13}O_2]^+$, 556 (100) $[M - C_{16}H_{30}O_2]^+$, 346 (57) $[M - C_{30}H_{42}NO_3]^+$, 167 (34) $[M - C_{40}H_{39}N_2O_6]^+$, 44 (39) $[C_2H_4O]^+$. HMRS: calcd. for $C_{53}H_{50}N_2O_6$ $[M]^+$ 810.367; found 810.368.

4′-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]biphenyl-4-carbaldehyde (1e): Column separation (silica gel; chloroform/ethanol, 40:1) of **1d** (300 mg, 0.370 mmol) gave an orange forerun. The intensely reddish-orange main fraction was concentrated, dissolved in the minimal amount of chloroform and precipitated with acetonitrile. Yield: 248 mg (0.323 mol, 87%). Bright light-red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/EtOH, 40:1) = 0.28. IR (ATR): $\tilde{\nu}$ = 2952.5 (m), 2924.0 (s), 2854.9 (m), 1691.9 (s), 1650.2 (vs), 1592.6 (s), 1577.7 (m), 1506.5 (w), 1456.1 (w), 1434.7 (w), 1403.6 (m), 1378.0 (w), 1332.6 (s), 1247.0 (m), 1214.8 (w), 1169.3 (m), 1125.8 (w), 1106.2 (w), 1003.6 (w), 987.8 (w), 849.6 (w), 808.2 (m), 782.0 (w), 748.8 (w), 740.3 (w), 606.5 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2 × CH_3], 1.18–1.38 (m, 16 H, 8 × CH_2), 1.83–1.91 (m, 2 H, β-CH_2), 2.21–2.29 (m, 2 H, β-CH_2), 5.15–5.22 (m, 1 H, α-CH), 5.46 (s, 2 H, NCH_2), 7.58–7.61 (m, 2 H, CH_{aryl}), 7.67–7.72 (m, 4 H, CH_{aryl}), 7.91–7.94 (m, 2 H, CH_{aryl}), 8.59–8.71 (m, 8 H, $CH_{perylene}$), 10.0 (s, 1 H, CHO) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.3, 22.8, 27.2, 29.4, 32.0, 32.6, 43.7, 55.1, 123.2, 123.5, 126.6, 126.8, 127.7, 127.8, 129.7, 129.8, 129.9, 130.5, 132.0, 135.3, 135.4, 137.7, 139.3, 147.0, 163.7, 192.1 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.2 (0.22), 490.4 (0.60), 527.0 nm (1.00); fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 535.2 (1.00), 576.5 nm (0.50); fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0132 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI^+, 70 eV): *m/z* (%) = 766 (21) $[M]^+$, 584 (100) $[M - C_{13}H_{26}]^+$, 346 (55) $[M - C_{28}H_{38}NO_2]^+$, 195 (14) $[C_{14}H_{11}O]^+$. HMRS: calcd. for $C_{53}H_{50}N_2O_6$ $[M]^+$ 766.340; found 766.339.

2-(2,2-Dimethoxyethyl)-9-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (1g): 9-(1-Hexylheptyl)-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]isoquinoline-1,3,8,10-tetraone (3.00 g, 5.23 mmol), aminoacetaldehyde dimethyl acetal (820 mg, 7.79 mmol) and imidazole (6 g) were allowed to react as was described for **1c** (yield: 3.3 g, 96%) and purified by column chromatography (silica gel; $CHCl_3$/acetic acid, 10:1). Yield: 2.96 g (86%). M.p. 274 °C. R_f (silica gel, $CHCl_3$) = 0.1. R_f (silica gel; $CHCl_3$/acetic acid, 10:1) = 0.87; R_f (alumina; $CHCl_3$) = 0.52. IR (KBr): $\tilde{\nu}$ = 3444 (s), 2965 (m), 2929 (m), 2857 (m), 1734 (w), 1700 (s), 1660 (s), 1636 (s), 1595 (s), 1580 (m), 1457 (m), 1437 (m), 1405 (s), 1345 (s), 1255 (m), 1124 (m), 1066 (m), 1050 (m), 860 (w), 810 (s), 746 (s) cm^{-1}. UV ($CHCl_3$): λ_{max} (ε) = 527 (84700), 489 (50800), 458 (19400) nm; fluorescence ($CHCl_3$): λ_{max} = 532, 570 nm. ^1H NMR ($CDCl_3$): δ = 0.81 (t, 6 H,2 CH_3), 1.28 (m_c, 16 H,8 CH_2), 1.88 (m_c, 2 H, 1 α-CH_2), 2.21 (m_c, 2 H, 1 α-CH_2), 3.46 (s, 3 H,1O CH_3), 3.49 (s, 3 H, 1O CH_3), 4.36 (m_c, 2 H,1 CH_2), 4.94 (t, 1 H, 1 CH), 5.20 (m, 1 H, CH), 8.40 (m_c, 8 H, aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.02, 22.59, 27.00, 29.23, 31.77, 32.38, 40.42, 53.29, 54.87, 100.35, 122.54, 122.68, 125.86, 129.01, 131.04, 133.73, 134.21, 163.00 ppm. MS (70 eV): *m/z* (%) = 662 (1), 661 (5), 660 (10) $[M]^+$, 629 (6) $[M - OCH_3]^+$, 572 (6) $[M - OCH_3 - C_3H_5O]^+$, 479 (4), 447 (5), 404 (7), 403 (5), 391 (5), 390 (9), $[M - OCH_3 - C_3H_5O - C_{13}H_{26}]^+$, 373 (4), 76 (3), 75 (100), 55 (2), 31 (4), 28 (1). $C_{41}H_{44}N_2O_6$ (660.4): calcd. C 74.55, H 6.66, N 4.24; found C 74.39, H 6.61, N 4.43.

[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra-[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-yl]acetaldehyde (1h): 1g (1.50 g, 2.27 mmol) was dispersed in 1,4-dioxane (50 mL), treated with 6 N HCl (20 mL), stirred at 40 °C for 4 h, cooled at room

temperature, diluted with distilled water (200 mL), collected by vacuum filtration (D4 glass filter), thoroughly washed with distilled water, dried in vacuo at 30 °C for 5 h, purified by column chromatography (silica gel; $CHCl_3$/acetic acid, 10:1) (removing a small forerun), concentrated in vacuo, precipitated with distilled water, collected by vacuum filtration, thoroughly washed until neutral and dried in vacuo at 40 °C for 5 h. Yield: 1.14 g (82%). M.p. 244 °C. R_f (silica gel; $CHCl_3$) = 0.08; R_f (silica gel; $CHCl_3$/acetic acid, 10:1) = 0.8. IR (KBr): $\tilde{\nu}$ = 3445 (m), 3095 (w), 2955 (m), 2928 (s), 2856 (m), 1735 (m), 1698 (s), 1658 (s), 1615 (w), 1595 (s), 1579 (m), 1508 (w), 1457 (m), 1437 (m), 1405 (s), 1375 (m), 1345 (s), 1302 (w), 1252 (m), 1174 (m), 1130 (w), 1107 (w), 1085 (w), 1055 (w), 1035 (w), 958 (w), 854 (m), 810 (s), 747 (m) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.85 (t, 6 H, 2 CH_3), 1.28 (m_c, 16 H, 8 CH_2), 1.92 (m_c, 2 H, 1 α-CH_2), 2.24 (m_c, 2 H, 1 α-CH_2), 5.05 (s, 2 H, 1 CH_2), 5.19 (m_c, 1 H, CH), 8.25 (m_c, 8 H, perylene), 9.79 (s, 1 H, aldehyde) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.03, 22.59, 27.00, 29.23, 31.77, 37.37, 54.93, (C-1 1-hexylheptyl), 49.61 (CH_2), 122.06, 122.48, 123.00, 125.75, 125.86, 129.01, 129.12, 131.19, 133.46, 134.54, (C-10 perylene), 162.47 (C=O), 194.03 (aldehyde) ppm. UV ($CHCl_3$): λ_{max} (ε) = 527.3 (91500), 490.1 (58200), 458.7 (26900) nm; fluorescence ($CHCl_3$): λ_{max} = 535, 574 nm. MS (70eV): *m*/*z* (%) = 616 (5), 615 (22), 614 (52) $[M]^+$, 597 (8), 4451 (5), 434 (18), 433 (53), 432 (50), 415 (6), 406 (7), 405 (35), 404 (100), 403 (9), 390 (7), 376 (8), 359 (8), 345 (5), 125 (3), 111 (6), 97 (9), 73 (10), 57 (14), 41 (9), 28 (7).

4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d*′*e*′*f*′]diisoquinolin-2-yl]butyraldehyde (1i): 9-(1-Hexylheptyl)-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]-isoquinoline-1,3,8,10-tetraone (3.30 g, 5.75 mmol), 4-aminobutyraldehyde diethyl acetal (1.85 g, 11.5 mmol) and imidazole (5 g) were allowed to react as was described for **1c** (400 mL of ethanol and 400 mL of 2 N HCl, yield: 3.45 g, 93%) and purified by column chromatography (silica gel; $CHCl_3$/acetic acid, 10:1). Yield: 2.72 g (74%). M.p. 257 °C, R_f (silica gel; $CHCl_3$/acetic acid, 10:1) = 0.53; R_f (silica gel; $CHCl_3$) = 0.01. IR (KBr): $\tilde{\nu}$ = 2955 (m), 2928 (m), 2855 (m), 1697 (s), 1658 (s), 1616 (w), 1595 (s), 1579 (m), 1506 (w), 1457 (w), 1440 (m), 1405 (s), 1343 (s), 1287 (w), 1249 (m), 1171 (w), 1135 (w), 1110 (w), 1060 (w), 850 (w), 810 (s), 745 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.9 (t, 6 H, 2 CH_3), 1.25 (m_c, 16 H,8 CH_2), 1.95 (m_c, 2 H, α-CH_2), 2.10 (m_c, 2 H, CH_2), 2.22 (m_c, 2 H, α-CH_2), 2.6 (m_c, 2 H, CH_2), 4.18 (m_c, 2 H, CH_2), 5.18 (m_c,1 H, CH), 8.2 (m_c, 8 H, aromatic), 9.8 (t, 1 H, CHO) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.03, 22.58, 26.64, 29.22, 30.86, 31.77, 32.36, 39.65, 41.38, 54.88, 122.48, 122.56, 122.60, 122.76, 129.15, 130.61, 130.90, 131.34, 133.50, 133.60, 133.87, 134.18, 162.91, 162.99, 201.23 ppm. UV ($CHCl_3$): λ_{max} (ε) = 526 (85900), 489 (51800), 458 (19200) nm; fluorescence ($CHCl_3$): λ_{max} = 532, 572 nm. MS (70 eV): *m*/*z* (%) = 644 (0.6), 643 (2), 642 (6) $[M]^+$, 614 (5), 588 (3), 587 (13), 586 (30), 569 (4), 461 (4) $[M - C_{13}H_{26}]^+$, 433 (5), 432 (12), 415 (4), 406 (14), 405 (53), 404 (100) $[M - C_{13}H_{26} - C_3H_4O]^+$, 390 (10) $[M - C_{13}H_{26} - C_3H_4O - CH_2]^+$, 359 (6), 69 (5), 55 (8), 44 (12), 41 (7), 28 (4). $C_{41}H_{42}N_2O_5$ (642.4): calcd. C 76.64, H 6.53, N 4.34; found C 76.75, H 6.36, N 4.37.

4-(1,3,8,10-Tetraoxo-2-benzopyrano[6′,5′,4′:10,5,6]anthra[2,1,9-*def*]isoquinolin-9-yl)butyraldehyde: 1,3-Dioxo-1*H*,3*H*-perylo-[3,4-*cd*]pyran-8,9-dicarboxylic acid, monopotassium salt[19] (4.50 g, 10.0 mmol) was added with stirring and cooling (0 °C) to a solution of 4-aminobutyraldehyde diethyl acetal in distilled water (50 mL), stirred at room temperature for 2 h, then at 90 °C for 2 h, quenched by the addition of 2 N HCl (50 mL), allowed to stand for 1 h, collected by vacuum filtration, thoroughly washed with distilled water, treated with 10% KOH (50 mL) at 90 °C for 1 h, collected by vacuum filtration, washed with 8% KCl and 2% K_2CO_3 until colourless washings, dissolved in hot distilled water (500 mL), filtered though a D4 glass filter to remove solids, precipitated by acidifying with concd. HCl, collected by vacuum filtration (D4 glass filter), thoroughly washed with distilled water, dried in air at 100 °C for 8 h (yield: 3.8 g, 82%), and extractively recrystallised[20] from chloroform. Yield: 3.0 g (65%). R_f (silica gel; $CHCl_3$/acetic acid, 10:1) = 0.61. IR (KBr): $\tilde{\nu}$ = 3450 (w), 3090 (w), 2962 (w), 2915 (w), 2890 (w), 1796 (s), 1734 (s), 1698 (s), 1658 (s), 1616 (w), 1594 (s), 1580 (m), 1505 (w), 1455 (w), 1435 (w), 1405 (m), 1355 (w), 1321 (m), 1298 (m), 1271 (w), 1243 (w), 1151 (w), 1125 (m), 1069 (w), 1044 (w), 1025 (m), 855 (w), 810 (s), 739 (m) cm^{-1}. UV ($CHCl_3$): λ_{max} (ε) = 526 (80200), 489 (50400), 459 (18900) nm. MS (70 eV): *m*/*z* (%) = 462 (2), 461 (6) $[M]^+$, 433 (36) $[M - CO]^+$, 417 (7), 416 (23), 413 (16), 404 (10), 392 (22), 391 (38) $[M - C_4H_6O]^+$, 374 (6), 345 (12), 341 (7), 333 (6), 320 (5), 319 (14), 274 (8), 248 (17), 124 (13), 64 (9), 44 (100), 36 (16), 28 (27). $C_{28}H_{15}NO_6$ (461.4): calcd. C 72.88, H 3.28, N 3.04; found C 72.10, H 3.32, N 2.87.

2-(1-Hexylheptyl)-9-[4-(phenyliminomethyl)benzyl]anthra[2,1,9-*def*:6,5,10-*d*′*e*′*f*′]diisoquinoline-1,3,8,10-tetraone (12a). Method 1: 1c (35.0 mg, 50.7 μmol) was dissolved in chloroform (3 mL), treated with $MgSO_4$ (300 mg) and dropwise with freshly distilled aniline (0.462 mL, deep-violet solution), heated at 60 °C for 3 h, stirred at room temperature for 12 h, separated from the solid by filtration (washed with chloroform until colourless), precipitated with methanol, collected by vacuum filtration and dried in air at 110 °C for 16 h. Yield: 25.0 mg (32.6 μmol, 64%). **Method 2: 1c** (45.0 mg, 65.1 μmol) was dissolved in chloroform (5 mL), treated with molecular sieves (4 Å; 1.00 g) and with freshly distilled aniline (0.593 mL, 65.1 μmol), stirred at room temperature for 12 h, separated from the solid by filtration, concentrated in vacuo, dissolved in a small amount of chloroform and precipitated with methanol. Yield: 38.0 mg (49.6 μmol, 76%). **Method 3: 1c** (17.0 mg, 24.6 μmol) was dissolved in freshly distilled aniline (3 mL), treated with $MgSO_4$ (300 mg), stirred at room temperature for 12 h, separated from the solid by filtration and precipitated with methanol. Yield: 16.0 mg (23.2 μmol, 94%). Red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.22. IR (ATR): $\tilde{\nu}$ = 2954.1 (m), 2923.6 (s), 2855.4 (m), 1694.4 (s), 1648.5 (vs), 1592.5 (s), 1577.1 (m), 1506.6 (w), 1484.0 (w), 1434.8 (m), 1402.9 (m), 1335.3 (s), 1301.6 (w), 1248.8 (m), 1170.2 (m), 1124.1 (w), 1105.6 (w), 980.4 (w), 912.1 (w), 846.5 (w), 808.5 (m), 794.5 (w), 780.2 (w), 759.1 (w), 743.1 (m), 693.5 (w), 644.5 (w), 620.2 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 0.83 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2 × CH_3], 1.18–1.39 (m, 16 H, 8 × CH_2), 1.85–1.92 (m, 2 H, β-CH_2), 2.20–2.30 (m, 2 H, β-CH_2), 5.16–5.22 (m, 1 H, α-CH), 5.44 (s, 2 H, NCH_2), 7.15–7.23 (m, 3 H, CH_{aryl}), 7.34–7.39 (m, 2 H, CH_{aryl}), 7.64–7.68 (m, 2 H, CH_{aryl}), 7.85–7.88 (m, 2 H, CH_{aryl}), 8.41 (s, 1 H, CHN), 8.49–8.69 (m, 8 H, CH_{aryl}) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.3, 22.8, 27.2, 29.5, 32.0, 43.8, 55.1, 121.1, 123.1, 123.2, 123.4, 126.1, 126.5, 126.6, 129.2, 129.3, 129.6, 129.7, 131.9, 134.4, 135.1, 135.8, 140.6, 152.3, 160.1, 163.5 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.6 (0.22), 490.8 (0.60), 527.6 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 534.0 (1.00), 577.0 (0.50) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0157 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d*′*e*′*f*′]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 765 (20) $[M]^+$, 583 (100) $[M - C_{13}H_{26}]^+$, 346 (11) $[M - C_{28}H_{39}N_2O]^+$. HMRS: calcd. for $C_{51}H_{47}N_3O_4$ $[M]^+$ 765.357; found 765.358.

2-(1-Hexylheptyl)-9-{4-[4-(phenyliminomethyl)phenyl]benzyl}-anthra[2,1,9-*def*:6,5,10-*d*′*e*′*f*′]diisoquinoline-1,3,8,10-tetraone (13a): **1e** (28.0 mg, 36.5 μmol), freshly distilled aniline (0.330 mL,

36.5 μmol), chloroform (5 mL) and $MgSO_4$ (400 mg) were allowed to react analogously to the procedure used to prepare **12a** (5 h, 60 °C). Yield: 22.0 mg (72%). Red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/EtOH, 40:1) = 0.23. IR (ATR): $\tilde{\nu}$ = 2953.8 (m), 2922.9 (s), 2854.8 (m), 1695.4 (s), 1655.6 (vs), 1593.7 (s), 1577.5 (m), 1554.6 (w), 1496.0 (w), 1483 (w), 1435.7 (m), 1379.2 (w), 1336.3 (s), 1249.9 (m), 1171.1 (m), 1123.5 (w), 1107.9 (w), 981.8 (w), 910.1 (w), 849.9 (w), 838.2 (w), 808.1 (m), 782.5 (w), 768.2 (w), 747.7 (m), 723.5 (w), 693.4 (w), 634.2 (w), 588.7 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2×CH_3], 1.18–1.38 (m, 16 H, 8×CH_2), 1.83–1.91 (m, 2 H, β-CH_2), 2.20–2.29 (m, 2 H, β-CH_2), 5.17–5.22 (m, 1 H, α-CH), 5.47 (s, 2 H, NCH_2), 7.20–7.25 (m, 3 H, CH_{aryl}), 7.37–7.41 (m, 2 H, CH_{aryl}), 7.59–7.63 (m, 2 H, CH_{aryl}), 7.65–7.73 (m, 4 H, CH_{aryl}), 7.91–7.96 (m, 2 H, CH_{aryl}), 8.47 (s, 1 H, CHN), 8.60–8.73 (m, 8 H, $CH_{perylene}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.3, 22.8, 27.2, 29.4, 32.0, 32.6, 43.7, 55.0, 121.1, 123.2, 123.3, 123.5, 126.2, 126.6, 127.5, 127.6, 129.4, 129.5, 129.8, 129.9, 132.0, 134.5, 135.2, 135.4, 137.1, 139.9, 143.9, 152.3, 160.1, 163.7 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.6 (0.21), 490.8 (0.59), 527.6 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 536.0 (1.00), 578.8 (0.38), 629.0 (0.12) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0198 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 841 (34) $[M]^+$, 659 (100) $[M - C_{13}H_{26}]^+$, 346 (49) $[M - C_{34}H_{43}N_2O]^+$, 104 (25) $[C_7H_6N]^+$. HMRS: calcd. for $C_{57}H_{51}N_3O_4$ $[M]^+$ 841.388; found 841.387.

2-[4-(Butyliminomethyl)benzyl}-9-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (12b): 1c (30.0 mg, 43.4 μmol), 1-butylamine (0.430 mL, 43.4 μmol), chloroform (5 mL) and $MgSO_4$ (400 mg) were allowed to react analogously to the procedure used to prepare **12a** (4 h, 50 °C). Yield: 14.0 mg (43%). Red solid, m.p. 245 °C. R_f (silica gel; $CHCl_3$/EtOH, 40:1) = 0.22. IR (ATR): $\tilde{\nu}$ = 2955.1 (m), 2924.0 (s), 2855.5 (s), 1693.9 (s), 1647.0 (vs), 1592.7 (s), 1576.5 (s), 1506.4 (w), 1458.0 (w), 1435.2 (m), 1402.9 (m), 1335.0 (s), 1303.2 (w), 1249.0 (m), 1170.4 (m), 1125.6 (w), 1106.3 (w), 983.0 (w), 849.6 (w), 809.4 (m), 794.8 (w), 779.4 (w), 743.9 (m), 631.0 (w), 586.8 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2×CH_3], 0.91 [t, ^3J(H,H) = 7.4 Hz, 3 H, $CH_{3,butyl}$], 1.18–1.38 (m, 18 H, 9×CH_2), 1.61–1.68 (m, 2 H, $CH_{2,butyl}$), 1.83–1.91 (m, 2 H, β-CH_2), 2.21–2.29 (m, 2 H, β-CH_2), 3.58 [t, ^3J(H,H) = 6.9 Hz, 2 H, $NCH_{2,butyl}$], 5.15–5.22 (m, 1 H, α-CH), 5.42 (s, 2 H, NCH_2), 7.58–7.64 (m, 2 H, CH_{aryl}), 7.66–7.72 (m, 2 H, CH_{aryl}), 8.22 (s, 1 H, CHN), 8.56–8.72 (m, 8 H, $CH_{perylene}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.1, 14.3, 20.6, 22.8, 27.1, 29.4, 29.9, 32.0, 32.6, 33.2, 43.8, 55.0, 123.2, 123.5, 123.6, 126.6, 126.7, 129.5, 129.6, 129.8, 130.3, 131.9, 132.1, 135.2, 163.6 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.4 (0.22), 490.8 (0.60), 527.4 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 536.0 (1.00), 577.2 (0.50) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0257 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 745 (24) $[M]^+$, 564 (25) $M - C_{13}H_{26}]^+$, 346 (18) $[M - C_{27}H_{43}N_2O]^+$, 173 (29) $[M - C_{37}H_{36}N_2O_4]^+$, 130 (100) $[C_9H_8N]^+$. HMRS: calcd. for $C_{49}H_{51}N_3O_4$ $[M]^+$ 745.388; found 745.383.

2-{4-[4-(Butyliminomethyl)phenyl]benzyl}-9-(1-hexylheptyl)anthra-[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (13b): 1e (28.0 mg, 36.5 μmol), 1-butylamine (0.700 mL, 70.4 μmol), chloroform (5 mL) and $MgSO_4$ (400 mg) were allowed to react analogously to the procedure used to prepare **12a** (12 h, room temperature, precipitation with acetonitrile). Yield: 17.0 mg (20.7 μmol, 59%). Dark-red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/EtOH, 40:1) = 0.23. IR (ATR): $\tilde{\nu}$ = 2953.3 (m), 2924.2 (s), 2854.9 (m), 1694.4 (s), 1653.1 (vs), 1592.7 (s), 1577.7 (m), 1496.6 (w), 1456.9 (w), 1434.6 (m), 1403.4 (m), 1377.8 (w), 1332.4 (vs), 1248.4 (m), 1216.3 (w), 1170.2 (m), 1124.2 (w), 1106.1 (w), 1004.0 (w), 982.9 (w), 851.3 (w), 808.1 (m), 782.4 (w), 747.7 (w), 665.7 (w), 636.1 (w) cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.82 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2×CH_3], 0.94 [t, ^3J(H,H) = 7.4 Hz, 3 H, $CH_{3,butyl}$], 1.18–1.43 (m, 18 H, 9×CH_2), 1.65–1.71 (m, 2 H, $CH_{2,butyl}$), 1.83–1.91 (m, 2 H, β-CH_2), 2.21–2.29 (m, 2 H, β-CH_2), 3.62 [t, ^3J(H,H) = 6.8 Hz, 2 H, $NCH_{2,butyl}$], 5.15–5.22 (m, 1 H, α-CH), 5.45 (s, 2 H, NCH_2), 7.56–7.61 (m, 4 H, CH_{aryl}), 7.62–7.68 (m, 2 H, CH_{aryl}), 7.74–7.77 (m, 2 H, CH_{aryl}), 8.28 (s, 1 H, CHN), 8.59–8.72 (m, 8 H, $CH_{perylene}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C, TMS): δ = 14.1, 14.3, 20.7, 22.8, 27.2, 29.4, 32.0, 32.6, 33.2, 43.7, 55.0, 61.8, 123.2, 123.3, 123.5, 126.6, 126.8, 127.4, 127.5, 128.7, 129.7, 129.8, 131.9, 135.2, 135.6, 136.8, 140.1, 143.0, 160.5, 163.7 ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.8 (0.22), 490.8 (0.60), 527.6 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 534.5 (1.00), 576.0 (0.50) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0167 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 821 (22) $[M]^+$, 640 (38) $[M - C_{13}H_{26}]^+$, 374 (28) $[M - C_{31}H_{47}N_2]^+$, 346 (60) $[M - C_{32}H_{47}N_2O]^+$, 206 (100) $[M - C_{40}H_{43}N_2O_4]^+$, 167 (49) $[C_{13}H_{11}]^+$, 84 (70) $[C_5H_{10}N]^+$. HMRS: calcd. for $C_{55}H_{55}N_3O_4$ $[M]^+$ 821.419; found 821.418.

4-[(1-{4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]phenyl}-methylidene)amino]benzoic Acid (12c): 1c (25.0 mg, 36.2 μmol) was dissolved in a mixture of chloroform/ethanol (10:4, 5 mL), treated with $MgSO_4$ (400 mg) and with 4-aminobenzoic acid (20.0 mg, 146 μmol), heated at 70 °C for 1 h, stirred at room temperature for 12 h, separated from the solid by filtration, concentrated, dissolved in chloroform (low solubility) and precipitated with acetonitrile. Yield: 11.0 mg (38%). Dark-red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.09; R_f (silica gel; $CHCl_3$/ethanol, 10:1) = 0.57. IR (ATR): $\tilde{\nu}$ = 3307.5 (w), 2066.0 (w), 2951.8 (m), 2924.1 (s), 2854.7 (m), 1693.7 (s), 1651.2 (vs), 1592.4 (vs), 1577.0 (s), 1506.9 (w), 1435.1 (m), 1403.8 (m), 1378.2 (w), 1333.4 (vs), 1247.8 (m), 1167.9 (m), 1125.7 (w), 1103.5 (w), 1013.9 (w), 981.5 (w), 889.2 (w), 851.0 (w), 808.6 (m), 775.1 (w), 743.8 (w), 696.9 (w), 625.1 (w) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.83 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2×CH_3], 1.16–1.39 (m, 16 H, 8×CH_2), 1.76–1.98 (m, 2 H, β-CH_2), 2.13–2.38 (m, 2 H, β-CH_2), 5.10–5.27 (m, 1 H, α-CH), 5.49 (s, 2 H, NCH_2), 7.15–7.23 (m, 2 H, CH_{aryl}), 7.64–7.77 (m, 2 H, CH_{aryl}), 7.81–7.95 (m, 2 H, CH_{aryl}), 8.41 (s, 1 H, CHN), 8.60–8.83 (m, 8 H, $CH_{perylene}$) ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.8 (0.22), 491.0 (0.60), 527.6 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 534.8 (1.00), 578.0 (0.50) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0132 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m*/*z* (%) = 810 (23) $[M]^+$, 627 (100), $[M - C_{13}H_{26}]^+$, 346 (23) $[M - C_{29}H_{39}N_2O_3]^+$, 238 (17) $[M - C_{37}H_{35}N_2O_4]^+$, 137 (46) $[C_7H_7NO_2]^+$, 120 (44) $[C_7H_4O_2]^+$, 91 (23) $[C_6H_5N]^+$. HMRS: calcd. for $C_{52}H_{47}N_3O_6$ $[M]^+$ 809.346; found 809.348.

2-(4-{4-[(4-Carboxyphenyl)iminomethyl]phenyl}benzyl)-9-(1-hexyl-heptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone (13c): 1e (25.0 mg, 32.6 μmol), a mixture of chloroform/ethanol (10:4, 5 mL), $MgSO_4$ (400 mg) and 4-aminobenzoic acid (17.8 mg, 130 μmol) were allowed to react analogously to the procedure used

to prepare **13a** (dissolved in chloroform and precipitated with methanol, low solubility). Yield: 4.00 mg (14%). Dark-red solid, m.p. >250 °C. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.07; R_f (silica gel; $CHCl_3$/ethanol, 10:1) = 0.47. IR (ATR): $\tilde{\nu}$ = 3305.9 (br, w), 3068.3 (w), 2951.4 (m), 2924.7 (s), 2954.9 (m), 1693.5 (s), 1651.5 (vs), 1591.9 (vs), 1577.7 (s), 1505.5 (w), 1435.3 (w), 1403.9 (m), 1333.0 (vs), 1247.7 (m), 1167.2 (m), 1124.4 (w), 1104.9 (w), 1004.2 (w), 982.1 (w), 889.7 (w), 851.5 (w), 808.1 (m), 780.7 (m), 747.7 (m), 723.8 (w), 665.6 (w), 623.1 (w) cm^{-1}. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.8 (0.22), 490.8 (0.60), 527.6 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 535.5 (1.00), 578.8 (0.52), 627.5 (0.12) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0202 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. MS (DEI$^+$, 70 eV): *m/z* (%) = 886 (15) $[M]^+$, 703 (100), $[M - C_{13}H_{26}]^+$, 374 (21) $[M - C_{34}H_{43}N_2O_2]^+$, 346 (27) $[M - C_{35}H_{47}N_2O_3]^+$, 314 (30) $[M - C_{37}H_{35}N_2O_4]^+$, 137 (81) $[C_7H_7NO_2]^+$, 120 (90) $[C_7H_4O_2]^+$, 92 (23) $[C_6H_6N]^+$. HMRS: calcd. for $C_{58}H_{51}N_3O_6$ $[M]^+$ 885.378; found 885.379.

2-[(1-{4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]phenyl}-methylidene)amino]-3-phenylpropionic Acid (12d). Method 1: 1c (30.0 mg, 43.4 μmol) was dissolved in *N*-methylpyrrolidone (NMP, 20 mL) at 110 °C, treated with $MgSO_4$ (400 mg) and with L-phenylalanine (25.0 mg, 151 μmol) in NMP (10 mL, dissolved at 110 °C), stirred at 110 °C for 5 h and at room temperature for 12 h, separated from the solid by filtration, shaken with concentrated brine (100 mL) and extracted with toluene (100 mL). The combined organic phases were three times shaken with concentrated brine (100 mL each), dried with $MgSO_4$, concentrated in vacuo and purified by column chromatography (silica gel; toluene/ethanol, 10:1) (second fraction). Yield: about 25%. R_f (silica gel; $CHCl_3$/ethanol, 40:1) = 0.03; R_f (silica gel; $CHCl_3$/ethanol, 10:1) = 0.51. ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C, TMS): δ = 0.86 [t, ^3J(H,H) = 7.0 Hz, 6 H, 2×CH_3], 1.18–1.38 (m, CH_2, 16 H), 1.38 (m, 1.80–2.30 (m, 4 H, β-CH_2), 3.89 (m, 1 H, CH), 4.46 (m, 2 H, CH_2), 5.10–5.28 (m, 1 H, α-CH), 5.41 (s, 2 H, NCH_2), 6.70–7.34 (m, 5 H, CH_{aryl}), 7.53–7.83 (m, 4 H, CH_{aryl}), 8.24 (s, 1 H, CHN), 8.59–8.78 (m, 8 H, $CH_{perylene}$) ppm. UV/Vis ($CHCl_3$): λ_{max} ($E_{rel.}$) = 459.2 (0.22), 490.4 (0.61), 527.2 (1.00) nm; fluorescence ($CHCl_3$): λ_{max} ($I_{rel.}$) = 536.0 (1.00), 579.2 (0.53), 629.0 (0.13) nm; fluorescence quantum yield {$CHCl_3$, λ_{exc} = 490 nm, E_{490nm} = 0.0295 cm^{-1}, reference: 2,9-bis(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinoline-1,3,8,10-tetraone with Φ = 1.00}: Φ = 1.00. **Method 2: 1c** (30.0 mg, 43.4 μmol) was dissolved in dimethyl sulfoxide (DMSO, 20 mL) at 110 °C, treated with $MgSO_4$ (400 mg) and with L-phenylalanine in DMSO (25 mg in 10 mL DMSO), dissolved at 110 °C, stirred at 110 °C for 5 h and at room temperature for a further 12 h, separated from the solid by filtration, shaken with concentrated brine (100 mL) and extracted with toluene (100 mL). The combined organic phases were three times shaken with concentrated brine (100 mL each), dried with $MgSO_4$, concentrated in vacuo (reddish-violet powder) and purified by column chromatography (silica gel; toluene/ethanol, 10:1) (second fraction). Yield: ca. 25%.

2-[(1-{4′-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]biphenyl-4-yl}methylidene]amino}-3-phenylpropionic Acid (13d): 1e (30.0 mg, 39.1 μmol) in NMP (20 mL) and L-phenylalanine (22.6 mg, 137 μmol) in NMP (10 mL) were allowed to react as was described for the preparation of **12d** (reddish-violet powder).

Fluorescence Labelling of Catalase with 4-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]benzaldehyde (1c). Method 1: Compound **1c** (20 mg, 28.9 μmol) and a microspatulum of dicyclohexylcarbodiimide (DCC) were added with stirring to a mixture of bovine catalase (bison liver, 40.0 mg) and molecular sieves (4 Å; 300 mg) in NMP (20 mL) at 40 °C, stirred for 60 h, cooled to room temperature, collected by filtration (the NMP phase can be used for further labelling of peptides), washed with water and methanol, separated from molecular sieves by fractionated sedimentation from methanol and dried in air to give a red powder. The red coloration could not be removed, neither with water nor with organic solvents such as chloroform. **Method 2:** Compound **1c** (18.0 mg, 26.1 μmol) was allowed to react as was described in Method 1 at 50 °C for 30 min to give a reddish-orange material. The coloration could not be removed, neither with water nor with organic solvents such as chloroform. **Method 3:** Compound **1c** (10.0 mg, 14.5 μmol) was dissolved in DMSO (10 mL) at 50 °C and a microspatulum of dicyclocarbodiimide (DCC) was added with stirring to a mixture of bovine catalase (18.0 mg) and molecular sieves (4 Å; 300 mg) in DMSO and stirred at 50 °C for 5 h and further treated as was described for Method 1. A dark-red material was obtained the coloration of which could not be removed, neither with water nor with organic solvents such as chloroform. **Method 4:** A microspatulum of **1c** and bovine catalase were dispersed in NMP (5 mL) at 50 °C for 1 h giving uncoloured flakes of catalase. A microspatulum of DCC was added (red colouration within a few minutes). The mixture was further stirred at 50 °C for 1 h and further treated as was described for Method 1. A reddish-orange material was obtained the colouration of which could not be removed, neither with water nor with organic solvents such as chloroform.

Fluorescence Labelling of Catalase with 4′-[9-(1-Hexylheptyl)-1,3,8,10-tetraoxo-3,8,9,10-tetrahydro-1*H*-anthra[2,1,9-*def*:6,5,10-*d′e′f′*]diisoquinolin-2-ylmethyl]biphenyl-4-carbaldehyde (1e): A microspatulum of **1e** was allowed to react as was described for Method 2. A reddish-orange material was obtained the colouration of which could not be removed, neither with water nor with organic solvents such as chloroform.

Supporting Information (see footnote on the first page of this article): Synthesis and spectroscopic characterization of **3**, **4**, **5**, **6**, **9**, **10** and **11**.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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Received: March 15, 2007
Published Online: July 6, 2007

