π···π Interactions of Flavins, 3[1]

[3.3]Isoalloxazinophanes and Arene-Bridged Bis(isoalloxazines): Syntheses, Characterizations and Properties Related to Intramolecular Interactions

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In order to study the orientation and distance dependence of π···π interactions between flavins in various redox states, two approaches to bis(isoalloxazine) model compounds are presented: The [3.3]isoalloxazinophanes **2** and **4** and the rigid single-bridged 1,8-naphthalene- and 1,8-anthracene-linked bis(isoalloxazine) systems **6**, **8** and **26** were synthesized, characterized, and studied by spectroscopic methods and cyclic voltammetry. The experimental data are discussed in comparison with "monomeric" isoalloxazines and flexible single-chain trimethylene-linked bis(isoalloxazine) reference compounds. X-Ray structure analyses are reported for the 1,8-bis(isoalloxazinyl)-9,10-dihydroanthracene **26** and for **22** as an example of 1,8-disubstituted naphthalenes with strongly polar substituents comparable to **6**.

One of the most important features in flavin chemistry is the ability of the isoalloxazine system to exist in three different stable redox states: the flavoquinone, the flavosemiquinone radical and the flavohydroquinone. These redox states can be further modified by protonation or deprotonation, formation of hydrogen bonds or steric factors. For these reasons, the flavocoenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) play an essential role in biological processes as mediators between nicotinamide nucleotides as exclusive two-electron carriers and typical one-electron carriers such as ferredoxin iron sulfur clusters or metalloporphyrins. It has also been postulated that in the active site of some enzymes (*e. g.* NADPH-cytochrome P_{450}-reductase) two flavins act as a single redox-active unit[2].

The quinhydrone-like association of oxidized and reduced flavins was first discussed by Kuhn and Ströbele[3]. Although the spectroscopic properties of semireduced flavin systems have been studied extensively since then[4], the spatial arrangement favoring charge-transfer interactions and the exchange of redox equivalents by electron transfer is still a matter of controversial discussion[5]. In the past, there have been some attempts to mimic these interactions by open-chain trimethylene-linked bis(isoalloxazines)[6]. As a major disadvantage of such models, however, it must be realized that their steric structure due to the flexibility of the linking chain is not well-defined. Suitable model systems for determining the orientation and distance dependence of flavin-flavin interactions require clearly defined rigid geometries with sandwich-like flavin-flavin orientation as well as a controlled variation of interplanar distances by the choice of different spacer groups.

With the intention to contribute to the understanding of flavin coenzyme functions we tried to synthesize models meeting these requirements. Results referring to flavin-flavin interactions are described in this paper; in the following papers we report on an analogous approach in the attempt to synthesize models for flavin interactions with nicotinamide[7] and quinones[8].

Based on earlier extensive experiences of our group in using the "cyclophane concept" for studying intramolecular charge-transfer interactions[9], excimer interactions[10] and electron-transfer reactions[11] with regard to their distance and orientation dependences, the requirements mentioned above should be satisfied by incorporating two isoalloxazine units into cyclophane systems. Thus, our first synthetic goals were the isoalloxazinophanes **1** and **3** as well as their respective chiral isomers **2** and **4** with different π···π overlap as compared to **1** and **3**, respectively.

A complementary approach to well-defined flavin-flavin systems makes use of rigid arene spacers like naphthalene and anthracene to the 1- and 8-positions of which two isoalloxazine units are linked through their 10-positions. In principle, there might exist again two isomers each with different mutual flavin-flavin orientations – the *syn-anti* pairs **5**/**6** and **7**/**8**, respectively[12]. By comparing the corresponding compounds with naphthalene and anthracene spacers, we expected to derive evidence regarding the distance dependence of flavin-flavin interaction.

The synthetic program thus consisted of attempts to synthesize the bis(isoalloxazine) compounds **1** to **8**. In addition, as relevant reference compounds some simple isoalloxazines and single-bridged trimethylene-linked bis(isoalloxazines) were to be synthesized (see below).

Regarding the nomenclature it should be mentioned that the systematic name of isoalloxazine is "benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione". Flavins in the strict sense are the corresponding 7,8-dimethyl derivatives. For the cyclophanes **1**–**4** a modified "cyclophane nomenclature"[13] is used in which for reasons of comparability the normal atom numbering of isoalloxazines is retained.

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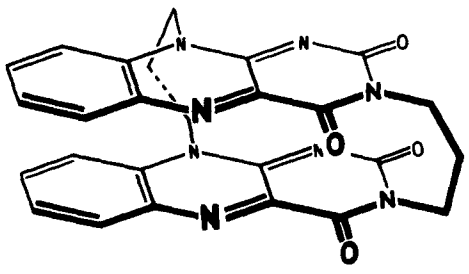

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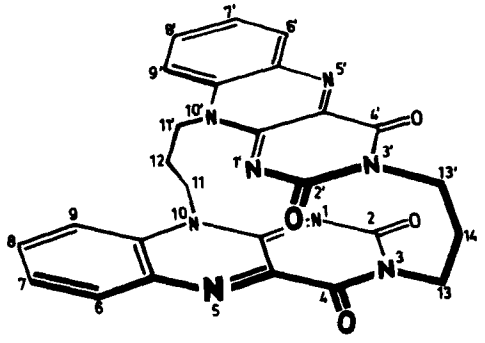

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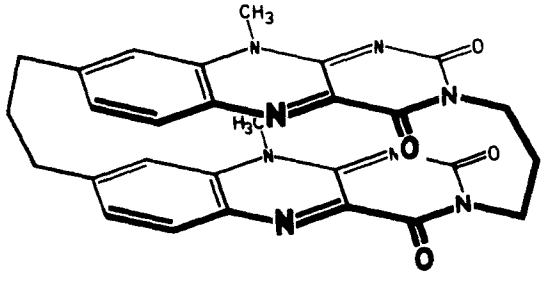

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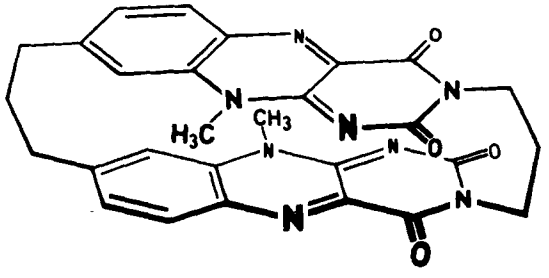

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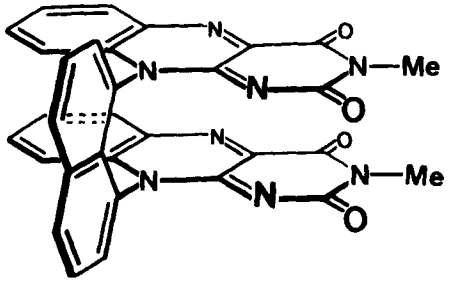

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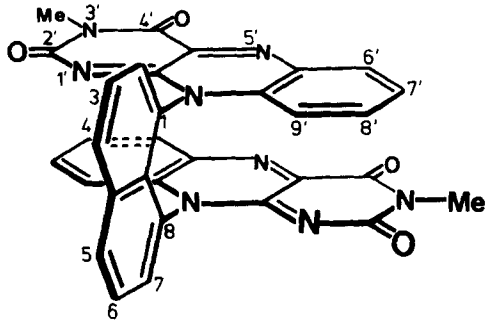

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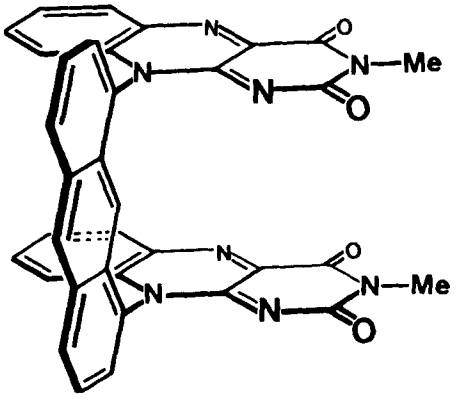

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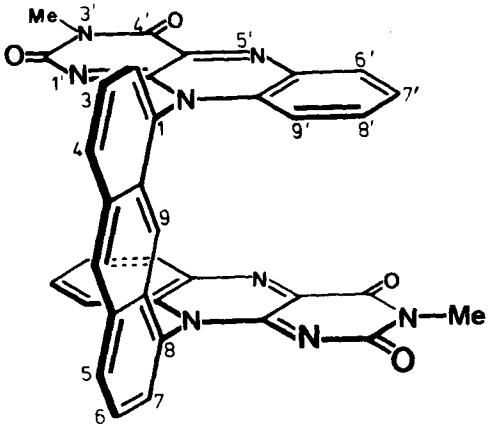
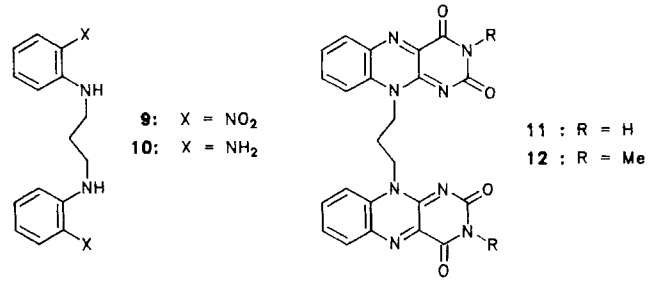


8

B

[3.3]Isoalloxazinophanes: Syntheses, Structures and Properties

[3.3](3,10)Isoalloxazinophanes (**1** and/or **2**): For the synthesis of **1** and/or **2**, trimethylene-*N*,*N*′-bis(2-nitroaniline) (**9**), prepared with slight modifications according to ref.[6a] (see Experimental), was catalytically hydrogenated (Pd/charcoal, acetic acid/boric acid, 70°C) to the corresponding diamino compound **10** which without isolation was treated with alloxan monohydrate (boric acid, acetic acid, 15h at 60–65°C, under nitrogen) to yield trimethylenebis(10-isoalloxazine) (**11**)[6a]. By *N*-methylation (iodomethane, DMF, potassium carbonate, 36h, 50°C, yield 70%) trimethylenebis[10-(3-methylisoalloxazine)] (**12**)[14] was obtained as an open-chain reference compound with the same substitution pattern as the [3.3](3,10)isoalloxazinophanes (**1/2**).



9: X = NO_2
10: X = NH_2

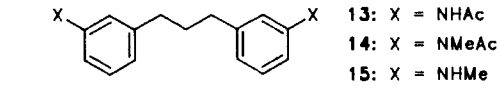
11 : R = H
12 : R = Me

The cyclization of **11** to the isoalloxazinophane structure was achieved by successive addition of 1,3-dibromopropane in the presence of an excess of potassium carbonate in dry DMF (102h, 55–60°C). By chromatography (silica gel; trichloromethane/formic acid/methanol) and subsequent crystallization from formic acid/water the cyclization product was obtained (yield 12%; fine yellow needles, m.p. 348–350°C, dec.). The [3.3](3,10)isoalloxazinophane structure is confirmed by elemental analysis, mass spectra and the absorption spectra which are very similar to those of **12** both of which showing the typical flavin bands with about doubled intensities. There was no evidence that in the cyclization reaction both isomers **1** and **2** were formed. All analytical and spectroscopic data including ^{1}H-NMR data (assignment based on NOE; see Experimental) were not sufficient to distinguish unambiguously between **1** and **2**, although from a thorough analysis of chemical shifts and splittings indications in favor of **2** were obtained[11]. The final assignment, however, was possible by ^{1}H-NMR spectrometry (360 MHz, $CDCl_3$/TFA, 1:1) in the presence of the chiral cosolvent (+)-2,2,2-trifluoro-1-(9′-anthryl)-ethanol (TFAE). With the chiral isomer **2**, (+)-TFAE was expected to form two diastereomeric associates which might result in splitting of the ^{1}H-NMR signals. In fact, in the presence of (+)-TFAE (ca. 790mmol/l) the signal of the 14-methylene protons is not only strongly highfield-shifted but also split into two multiplets of the same intensity and shape ($\Delta\delta$ = 0.06, at 303K); as to be expected this splitting increases with decreasing temperature ($\Delta\delta$ = 0.083, at 278K). With racemic TFAE under otherwise analogous conditions only one single multiplet (δ = 1.98–2.08) is observed for the 14-methylene protons. Obviously, the interaction of **2** with TFAE occurs preferentially at the site of the carbonyl-containing heterocycle; thus, the 3-N···3′-N trimethylene bridge is more exposed to TFAE than the 10-N···10′-N trimethylene bridge which within the central part of the molecule is more shielded and therefore its enantiotopic protons are not split by complexation with (+)-TFAE. On the basis of these experiments the chiral structure **2** for the cyclization product of **11** with 1,3-dibromopropane is well established.

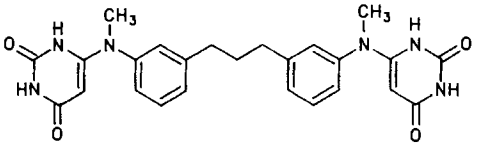
In contrast to **1** for which an approximately ecliptic orientation of the two isoalloxazine units was to be expected, in isomer **2** the overlap between the π systems of the subunits should be considerably reduced. Although, due to the unavailability of **1**, a comparison of overlap-dependent properties of the two isomers was not possible, some information with regard to this problem could be derived by comparing the spectroscopic properties of **2** with those of the open-chain analogue **12**. The UV/Vis absorption spectra of **2** and **12** display the typical flavin bands which are very similar to those of "monomeric" isoalloxazines with the exception of the nearly doubled absorption intensities [**2**: λ_{max} (nm; in brackets lg ε) = 269 (4.79), 341 (4.25), 430 (4.26); **12**: 265 (4.83), 338 (4.20), 434 (4.27), in ethylene glycol]. For studying the semireduced systems, solutions of **2** and **12** ($1.2 \cdot 10^{-4}$ M in ethylene glycol) were fully reduced by addition of aqueous sodium dithionite solution (0.475 M, under argon) and then partially reoxidized by shaking the tube with admission of air in small doses until the new absorptions at longer wavelengths reached their maximum intensity (neither the fully oxidized nor the completely reduced state of isoalloxazines show absorptions above 530nm). For **12** in the semireduced state a strong and very broad absorption band is observed with $\lambda_{max} \approx$ 770nm (lg $\varepsilon \approx$ 2.8) extending into the near infrared beyond 1100nm. This absorption is assigned to a quinhydrone-like system in which the trimethylene chain is folded into an arrangement where the isoalloxazine units approach each other toward a parallel sandwich-like orientation. Surprisingly at first sight, the semireduced isoalloxazinophane **2**, prepared in the same way, does not exhibit a typical charge-transfer absorption like **12**. It is true that there appears a new absorption, too, but this is observed in the range from 530 to 630nm and does not extend beyound 670nm (most likely this absorption is to be ascribed to the flavosemiquinone state of **2**). The difference compared to **12** obviously is due to the only partial overlap of the two isoalloxazine units of **2** and their fixation in an orientation which does not allow an overlap between the respective donor and acceptor regions. On the other hand, the more flexible open-chain bis(isoalloxazine) **12** may reach a conformation with favorable π···π overlap of donor and acceptor regions which for **2** is excluded by the rigid structure.

In the emission spectra both of **2** and **12**, in addition to the typical flavin phosphorescence, at very low temperature an unusually strong delayed fluorescence is observed which is attributed to an interaction of the two isoalloxazine units in their excited states (for experimental details and the discussion of these results see ref.[15]).

10,10'-Dimethyl[3.3](3,8)isoalloxazinophane (**3** and/or **4**): The synthesis aimed at the isoalloxazinophanes **3** and **4** started from 1,3-bis(3'-nitrophenyl)-2-propen-1-one prepared by condensation of 3-nitrobenzaldehyde with 3-nitroacetophenone[16]. Catalytic hydrogenation (Pd/charcoal, methanol, presence of catalytic amount of 4-toluenesulfonic acid, 18h, 4 bar, room temp.) and subsequent reaction with acetic anhydride (methanol, 2h at reflux) yielded 1,3-bis[3-(acetylaminophenyl)]-1-propanol. The hydrogenolytic reduction of the benzylic hydroxyl group was achieved by a second hydrogenation (Pd/charcoal, methanol, 4 bar, 24h, presence of 4-toluenesulfonic acid) yielding trimethylenebis(3-acetylaminobenzene) (**13**). With iodomethane in a phase-transfer system (dichloromethane/2N aqueous sodium hydroxide, tetra-*n*-butylammonium hydrogen sulfate, 3h reflux) the *N*-methylation product **14** was obtained, which was deacylated with potassium hydroxide in water/methanol (4h at reflux) to 1,1'-trimethylene-*N*,*N*'-bis(3-methylamino)benzene (**15**).



13: X = NHAc
14: X = NMeAc
15: X = NHMe



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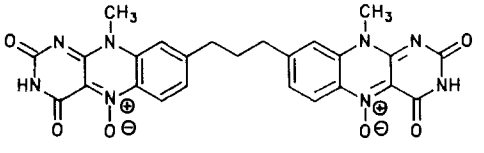

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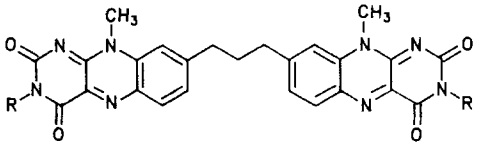
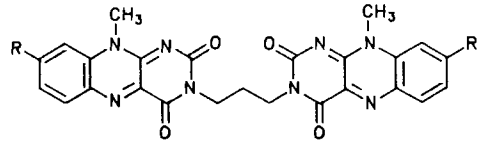


18: R = H
19: R = Me



20: R = H
21: R = Me

Starting from **15** the synthesis of the two flavin units made use of the nitrosative cyclization of 6-(*N*-alkylanilino)uracils introduced by F. Yoneda et al.[17]. For this purpose **15** and 6-chlorouracil were heated to 200°C, the reaction mixture was dissolved in ethanol, and the crude product purified by chromatography resulting in the isolation of **16** (m.p. 258–260°C, yield 64%). The cyclization of **16** was achieved by treatment with sodium nitrite in acetic acid (4h, room temp.) yielding the bis(isoalloxazine 5-oxide) **17** (m.p. 276°C; 48%). **17** was reduced by aqueous sodium dithionite solution (6h, room temp.) followed by reoxidation with hydrogen peroxide of the dihydroisoalloxazine units to trimethylenebis[8-(10-methylisoalloxazine)] (**18**: yellow crystals, m. p. 328–330°C; yield 41%). **18** as the basic primary product for the intended synthesis of the isoalloxazinophanes **3/4** and the corresponding open-chain system **19** were characterized by elemental analysis, mass spectra and completely assigned ^{1}H-NMR data (see Experimental).

For the final cyclization to the [3.3](3,8)isoalloxazinophane system, to a suspension of **18** and potassium carbonate in dry DMF at 55–60°C 12.5 equivalents of 1,3-diiodopropane were added within 120h (for details see Experimental). Chromatography (silica gel, trichloromethane/methanol, 9:1) yielded only one of the isoalloxazinophanes **3** and **4** as orange microcrystals (m.p. 368–370°C, dec.; yield 1.8%). High-resolution mass spectra were in accordance with both possible isomers **3** and **4**. The ^{1}H-NMR spectrum was fully assigned by COSY and by comparison with the reference bis(isoalloxazines) **19** and **20** (see Experimental). The ^{1}H-NMR data did not allow us to distinguish between the isomeric structures **3** and **4**. By addition of (+)-2,2,2-trifluoro-1-(9'-anthryl)ethanol as cosolvent, in contrast to **2**, no splitting of the 14,14'-H signal was observed as could be expected for the chiral isomer **4**. This negative result, however, was not to be valued as conclusive evidence in favor of the achiral isomer **3**. In fact, a one-dimensional rotating-frame Overhauser effect (ROE) spectrum showed a correlation with the signals of 9,9'-H (δ = 7.66) and 6,6'-H (δ = 8.30) on saturation of the 10,10'-methyl signal (δ = 4.48). This result indicates the presence of **4** since only in this chiral isomer the 10,10'- and 6,6'-positions are close enough for this kind of polarization transfer.

As reference systems, the open-chain bis(isoalloxazines) **19**, **20** and **21** were prepared for comparison with **4**; **19** was obtained by methylation of **18** with iodomethane (DMF, potassium carbonate, 50°C, 6h; yield 24%, yellow needles of m.p. 324°C). **20** and **21** are open-chain systems with the trimethylene chain linking 3-N and 3'-N of the isoalloxazine units, thus formig complementary analogues of the 10-C···10'-C-bridged isomers **18** and **19**, respectively. **20** was obtained by reaction of 10-methylisoalloxazine[18] with 1,3-iodopropane in the presence of excess potassium carbonate, and in analogy to the synthesis of **20** the corresponding bis(8,10-dimethylisoalloxazine) **21** was obtained from 8,10-dimethylisoalloxazine[17] (for details see Experimental).

The UV/Vis absorption spectra of the bis(isoalloxazines), measured for reasons of solubility in trifluoroacetic acid as mono-protonated species, show absorption wavelengths closely resembling the simple di- and trimethyl-substituted isoalloxazines with the corresponding alkyl-substitution pattern [3,8,10-trimethylisoalloxazine: λ_{max} (nm; in brackets lg ε) = 260 (4.52), 389 (4.42); **21**: 260 (4.72), 390 (4.65); **19**: 263 (4.77), 395 (4.73); **20**: 260 (4.82), 367 (4.55); **4**: 258 (4.60), 383 (4.40); in TFA]. The formally semireduced bis(isoalloxazines), obtained by treatment of **19**, **20** and **21** with half an equivalent of stannous chloride, show in TFA two absorption bands around 350 nm (lg $\varepsilon \approx$ 4.25–4.35) and 470nm (lg $\varepsilon \approx$ 4.15–4.30) most likely to be assigned to the protonated flavosemiquinone radical cations. On the

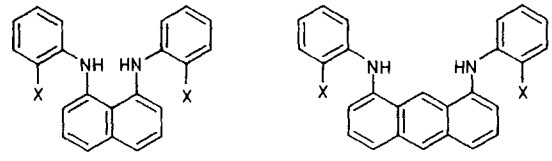
other hand, for the semireduced isoalloxazinophane **4** under the same conditions besides the two major absorptions at 345 nm (lg ε = 4.34) and 495 nm (lg ε = 4.23) an additional long-wavelength absorption centered at ca. 860 nm (lg ε = 3.54) and extending beyond 1000 nm was observed. This absorption may be assigned to an intramolecular charge-transfer transition although it has to be noted that formally semireduced flavins in solution in general do not exist as single well-defined species but as a rather complex system of molecules in various redox states.

Arene-Bridged Bis(isoalloxazines): Syntheses and Properties

With regard to flavin-flavin interactions the fixation of two isoalloxazine units in gradually varied distances was of special interest. The corresponding 1,8-disubstituted naphthalenes, biphenylenes and anthracenes offer a series in which the intramolecular distances between the isoalloxazine planes are expected to vary from about 2.5–3.5 Å in the naphthalene system to the order of 5 Å in the anthracene analogues, thus representing a distance range within which π···π interactions should be expected to differ considerably. Whereas the synthesis of biphenylene-spacered bis(isoalloxazines) has not yet been completed due to difficulties in building up the required 1,8-disubstituted biphenylene precursors[19], the bis(isoalloxazines) with naphthalene and anthracene spacers have been prepared and characterized.

1,8-Bis(3'-methylisoalloxazin-10'-yl)naphthalene (**5** and/or **6**): The synthesis started from 1,8-diaminonaphthalene which was treated with 2-fluoronitrobenzene (DMSO, potassium hydroxide, 4 h, 50°C, under argon) to yield, 1,8-bis(2'-nitrophenylamino)naphthalene (**22**). By catalytic hydrogenation of **22** (Pd/charcoal, acetic acid) 1,8-bis(2'-aminophenylamino)naphthalene (**23**) was obtained. Immediate reaction of **23** with alloxan hydrate (boric acid, acetic acid, 4 h, 20°C, under argon) yielded the corresponding bis(isoalloxazinyl)naphthalene. Due to its poor solubility this product was not purified and characterized but directly methylated (iodomethane, DMF, potassium carbonate, 20°C, 18 h) to 1,8-bis(3'-methylisoalloxazin-10'-yl)-naphthalene of which apparently only one of the two possible isomers **5** and **6** was obtained. Elemental analysis and mass spectrum, of course, would be consistent with either of these isomers, and ^{1}H-NMR analysis, too, does not allow a clear assignment to one of the two structures (for the respective data see Experimental).

^{1}H-NMR measurements in the presence of the chiral lanthanoid shift reagent Eu(TFC)$_3$[20], however, provided evidence in favor of the chiral *anti* isomer **6**; the addition of this shift reagent resulted in a splitting of the signal for the 3'-methyl protons [δ = 3.17, Δδ ≈ 0.01; 500 MHz, 305 K, $CDCl_3$, ca. 1 mg/ml Eu(TFC)$_3$]. The observed splitting especially of the 3'-methyl signal is in agreement with the expected coordination of the shift reagent to the carbonyl groups of the isoalloxazine systems. The exclusive (or at least predominant) formation of the *anti* isomer appears to be understandable on the basis of the interaction between the two closely neighboring isoalloxazine units in the 1,8-positions of naphthalene: the *syn* isomer with the eclipsed orientation should be destabilized by repulsive dipole-dipole interactions whereas in the *anti* isomer electron-donor and electron-acceptor regions are facing each other. The conclusiveness of this argumentation seemed limited at first sight by the X-ray structure analysis of **22** which proved for the crystalline state a *syn* structure with a nearly eclipsed arrangement of the polar substituents (see below). In solution, however, both conformers exist in an equilibrium; they can be identified by low-temperature ^{1}H-NMR which below 215 K shows two separated NH signals with δ = 9.25 and 9.93 of which the first one with the help of Eu(TFC)$_3$ was clearly assigned to the chiral *anti* isomer. The coalescence of these signals occurs at 245 K, resulting in a $\Delta G^{\neq}_{Tc}$ of 46 kJ · mol^{-1} for the *syn-anti* isomerization. From the intensities of the signals of the two species it can be concluded that the *anti* conformer of **22** is energetically slightly preferred (ΔG ≈ 400 J · mol^{-1}, 215 K). Of course, **22** as an equilibrating system of *syn-anti* isomers cannot strictly be compared with **6**, where due to the complete steric hindrance of the isomerization the isomer ratio of the product depends on the reaction kinetics of its formation.



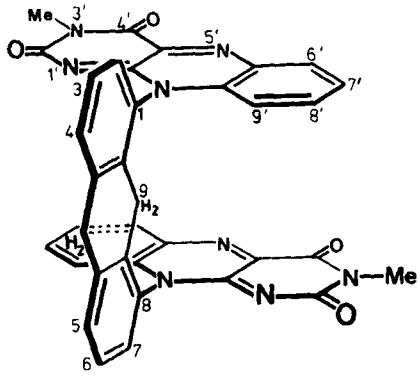
22: X = NO_2
23: X = NH_2

24: X = NO_2
25: X = NH_2

1,8-Bis(3'-methylisoalloxazin-10'-yl)anthracene (**7** and/or **8**) *and 1,8-Bis(3'-methylisoalloxazin-10'-yl)-9,10-dihydroanthracene* (**26**): In analogy to the corresponding naphthalene-spacered compound, for the synthesis of the anthracene-spacered bis(3'-methylisoalloxazine) system **7/8** 1,8-diaminoanthracene[21] was the key starting component which was prepared by reduction of 1,8-diaminoanthraquinone[22] to 1,8-diamino-9-anthrone (zinc, 25% aqueous ammonia, 2-propanol, 2 h, 90–120°C, under argon; yield 74%) and further reduction by sodium borohydride (1,4-dioxane, 18 h, 140°C, under argon; yield 92%). 1,8-Diaminoanthracene forms air-sensitive, bright yellow needles of m.p. 149–151°C [from petroleum ether (b.p. 40°C)/ethyl acetate]. Reaction of this diamino compound with 2-fluoronitrobenzene (DMSO, potassium hydroxide, 150 min, 20°C, under argon) yielded 1,8-bis(2'-nitrophenylamino)anthracene (**24**, red platelets, m.p. 290°C, dec.; yield 2.7%) which was catalytically hydrogenated (palladium/charcoal, DMF) to **25** which due to its high air-sensitivity was not isolated but directly treated with alloxan hydrate (boric acid, acetic acid, 4 h, 20°C) and *N*-methylated with iodomethane (DMF, potassium carbonate, 18 h, 20°C). Filtration of the crude product mixture from trichloromethane/methanol (10:1) on silica gel and subsequent MPLC (silica gel; trichloromethane/methanol, 40:1) resulted in the isolation of three isoalloxazine-containing compounds **A**, **B**, and **C**.

A (orange platelets crystallized from acetonitrile, m.p. 315°C, dec.; yield 8.6%) was identified by high-resolution FAB-mass determination and by its fully assigned ^{1}H-NMR spectrum as 1,8-bis(3′-methylisoalloxazin-10′-yl)anthracene. Of the two isomers **7** (*syn*) and **8** (*anti*) obviously only one compound was obtained as was the case for the naphthalene analogue (see above). Again the discrimination of the *syn-anti* isomers was possible by ^{1}H-NMR spectrometry in the presence of the chiral shift reagent Eu(TFC)$_3$[20]. In the ^{1}H-NMR spectrum [500 MHz, 305 K, CD_2Cl_2, ca. 1 mg/ml Eu(TFC)$_3$] the splitting of the 3′-methyl signal ($\Delta\delta \approx 0.02$) clearly indicates the presence of the chiral *anti* isomer **8**. A further interesting feature of the ^{1}H-NMR spectrum of **8** is the difference of 2.53 ppm between the 9- and 10-protons, which is due to the strong upfield shift of the 9-H signal to $\delta = 6.65$; this shift must be attributed to an anisotropic shielding effect of the two neighboring isoalloxazine systems.

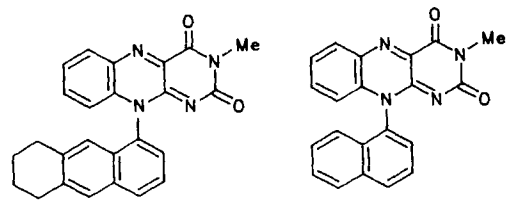
Based on the analytical data (see Experimental) compound **B** was identified as the 1,8-bis(3′-methylisoalloxazin-10′-yl)-substituted 9,10-dihydroanthracene **26**. Obviously, the catalytic hydrogenation of **24** leads to partial hydrogenation in the sensitive 9,10-positions of the anthracene unit. In this case, too, the application of the chiral shift reagent proved the presence of the chiral *anti* arrangement of the isoalloxazine systems. For **26** this structure was confirmed by an X-ray structure analysis the details of which are reported below.



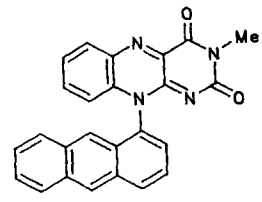
26

Compound **C**, the second by-product of the synthesis of **8**, was identified on the basis of its analytical and spectroscopic data (see Experimental) as 10-(5′,6′,7′,8′-tetrahydro-1′-anthryl)-3-methylisoalloxazine (**27**). The formation of **27**, in which one of the two lateral six-membered rings of the anthracene spacer is tetrahydrogenated, is not yet clear.

Further 10-Aryl-3-methylisoalloxazines: **27** can be considered as a mono(isoalloxazin-10-yl)arene. In connection with the problem of interactions between the two isoalloxazine units in **6**, **8** and **26**, further mono(isoalloxazinyl)-substituted naphthalenes and anthracenes were of interest as reference compounds without such interactions of the flavin-flavin type.



27 **28**



29

10-Phenyl-3-methylisoalloxazine had previously been synthesized[23]. For 10-(1′-naphthyl)isoalloxazine[24] and its 3-methyl derivative **28**[25] improved syntheses and complete spectroscopic data are reported in this paper. The corresponding 10-(1′-anthryl)-3-methylisoalloxazine (**29**) was prepared by starting from 1-aminoanthracene which was treated with 2-fluoronitrobenzene to yield 1-(2′-nitrophenylamino)anthracene. Reduction to 1-(2′-aminophenylamino)anthracene and condensation with alloxan hydrate/boric acid yielded 10-(1′-anthryl)isoalloxazine which was methylated to **29** (for synthetic and spectroscopic data see Experimental).

UV/Vis Spectra of Arene-Spacered Bis(isoalloxazines): The absorption spectra of the arene-bridged bis(isoalloxazines) **6**, **8** and **26** in their fully oxidized state are very similar to those of the reference compounds **28** and **29** [**6**: 264 (4.23), 348 (3.75), 460 (3.54); **8**: 259 (4.95), 345 (4.17), 438 (4.10); **26**: 264 (4.83), 340 (4.17), 442 (4.21); λ_{max}(nm), lg ε (in parentheses); 10^{-5}M solutions in ethylene glycol]. Only the naphthalene-spacered **6** with the shortest distance between the isoalloxazine units shows a bathochromic shift of 24 nm for the first absorption maximum as compared to **28** [λ_{max} = 436 (4.02)]. This shift is most likely due to intramolecular $\pi\cdots\pi$ interactions between the two isoalloxazine units although an effect of a distortion of the isoalloxazine chromophores by the sterical crowding of the neighboring 1,8-substituents cannot be excluded.

The formally semireduced state of these bis(isoalloxazines) was generated as described for **2** (see above). Whereas for **8** and **26** no additional long-wavelength absorptions are observed, again the naphthalene-spacered system **6** behaves differently: the semireduced **6** shows a new absorption band (extending from ca. 600 to 1200 nm with a strong maximum at 840 nm (lg $\varepsilon \approx 2.9$; in ethylene glycol). This absorption is most likely to be assigned to an intramolecular charge-transfer interaction between the dihydroisoalloxazine unit as electron donor and the isoalloxazine system as electron acceptor. The distance dependence of the charge-transfer absorption as derived from the comparison of **6** with **8** and

26 is well in agreement with our earlier results on [*n.n*]paracyclophane quinhydrones showing that beyond a donor-acceptor distance of about 400 pm significant charge-transfer absorptions are no longer observed[26].

Electrochemical Studies of Arene-Spacered Bis(isoalloxazines) by Cyclic Voltammetry: With regard to intramolecular redox reactions between isoalloxazines the influence of structure and distance on redox potentials was of interest. Redox potentials were measured in acetonitrile ("glassy carbon electrode" vs. Ag/AgCl; details see Experimental), and they are cited with reference to ferrocene.

For flavins, the reductive scan generally shows two one-electron reductions: the first reduction step ($FL/FL^{\bullet -}$) is reversible, the second ($FL^{\bullet -}/FL^{2-}$) is irreversible due to the protonation of the strongly basic dihydroflavin dianion by traces of water in the solvent. Since for the second reduction step only the cathodic peak potential is observed, the given values do not correspond to the thermodynamic redox potential. Therefore, the discussion is focused on the reversible first reduction step.

The reference compound 10-phenyl-3-methylisoalloxazine shows a reversible reduction at −1.13 V and the cathodic peak potential of the second step at −2.00 V. The naphthyl- and tetrahydroanthryl-substituted isoalloxazines **28** and **27** display a similar behavior (first reduction potentials at −1.13 V, cathodic peak for second reduction step at −2.09 V and −2.10 V, resp.). The cyclovoltammogram of the anthryl-substituted **29** shows, in addition to the two isoalloxazine reduction steps (−1.12 and −2.09 V), the reversible one-electron reduction of the anthracene subunit at −2.36 V.

For the naphthalene-spacered bis(isoalloxazine) **6** one reversible one-electron reduction at −1.00 V, two unresolved one-electron reductions at −1.30 V, and the cathodic peak potential of the one-electron step at −2.45 V corresponding to the normal second flavin reduction step were observed. In the potential area of the first flavin reduction ($FL/FL^{\bullet -}$) altogether three electrons are transferred to two isoalloxazine subunits. It is remarkable that the redox potential of the first reduction is shifted anodically by 0.30 V compared to the following reduction steps and by 0.13 V with reference to the corresponding potential of **28**. The fourth electron is transferred at the potential of the second flavin reduction ($FL^{\bullet -}/FL^{2-}$).

The anthracene- and dihydroanthracene-spacered systems **8** and **26** show three unresolved reversible one-electron reductions at −1.15 and −1.18 V, respectively. The fourth reduction step occurs at −2.24 and −2.29 V (irrev.). In the case of **8** at −2.41 V the reversible one-electron reduction of the anthracene spacer is observed. The peak current of this transition was used as an internal standard for the determination of the number of electrons transferred in the other transitions. In all bis(isoalloxazines) a change in the voltage increase rate did not significantly affect the electrochemical characteristics.

Thus, the transfer of three electrons at the potential range of the first flavin reduction occurs in all three bis(isoalloxazines) **6**, **8** and **26**. In the case of **6**, however, the first electron is accepted more easily than the following ones. A reason for the specific behavior of **6** could be the extreme steric repulsion between the isoalloxazine subunits in the naphtalene-spacered system. While flavoquinones are rigidly planar, the flavosemiquinone radical anion might be more easily bent along the N(5)···N(10) axis. Accordingly, the transfer of the first electron to **6** reduces the steric strain in the resulting flavoquinone-flavosemiquinone radical anion system by increasing its conformational flexibility and this loss of steric energy would shift the first reduction potential to a more positive value. Analogous effects have been discussed to explain the wide range of flavin redox potentials at the active site of flavoenzymes[27]. In the sterically less crowded bis(isoalloxazines) **8** and **26** no such effect is observed.

For the bis(isoalloxazines) the transfer of three electrons at potentials in the range of the regular first flavin reduction potentials is not yet fully understood. It seems likely that an intramolecular electron transfer in the semireduced redox state is involved. After the transfer of one electron each to the two isoalloxazine subunits at about the ($FL/FL^{\bullet -}$) potential an electron transfer would lead to a quinhydrone-like flavin-flavohydroquinone dianion system. A third electron then must be assumed to be transferred to the regenerated isoalloxazine subunit at about the same potential.

X-Ray Structure Analyses of Compounds Relevant to Arene-Spacered Bis(isoalloxazine) Systems

Unfortunately, neither the [3.3]isoalloxazinophanes **2** and **4** nor the naphthalene- and anthracene-spacered bis(isoalloxazines) **6** and **8** yielded crystals suitable for X-ray structure analyses. A structure analysis was achieved, however, for the third compound in the series of arene-spacered bis(isoalloxazines), the 9,10-dihydroanthracene analogue **26** (for crystallographic data see Experimental). In addition, the structure of 1,8-bis(2′-nitrophenylamino)naphthalene (**22**) has been solved; as was discussed above, **22** is a key precursor for the synthesis of **6**, and its structure was of interest with regard to the interaction of large substituents with highly polar groups in the two 1,8-positions of naphthalene.

anti-1,8-Bis(3′-methylisoalloxazin-10′-yl)-9,10-dihydroanthracene (**26**): In contrast to the expectations for the molecular structures of **6** and **8**, the isoalloxazine units in **26** are not oriented in a parallel arrangement to each other. The 9,10-dihydroanthracene spacer including the lateral aromatic rings is bent along the C(9)···C(10) axis by 34.4°. With regard to these lateral rings, on the other hand, the perfectly planar isoalloxazine units are nearly orthogonal leading to an interplanar angle between the two isoalloxazine units of 35.5°. The center-to-center distance between the isoalloxazines amounts to 540 pm, and there is a great difference between the shortest distance [C(8′)···C(2″): 336 pm] and the longest distance [N(3′)···C(7″): 747 pm] (see Figure 1). The isoalloxazine units are arranged to each other in an *anti* orientation. With the exception of the feasibility of slight rotational motions around the C(1)−N(10′) and C(8)−N(10″) bonds the molecule of **26** has a rather

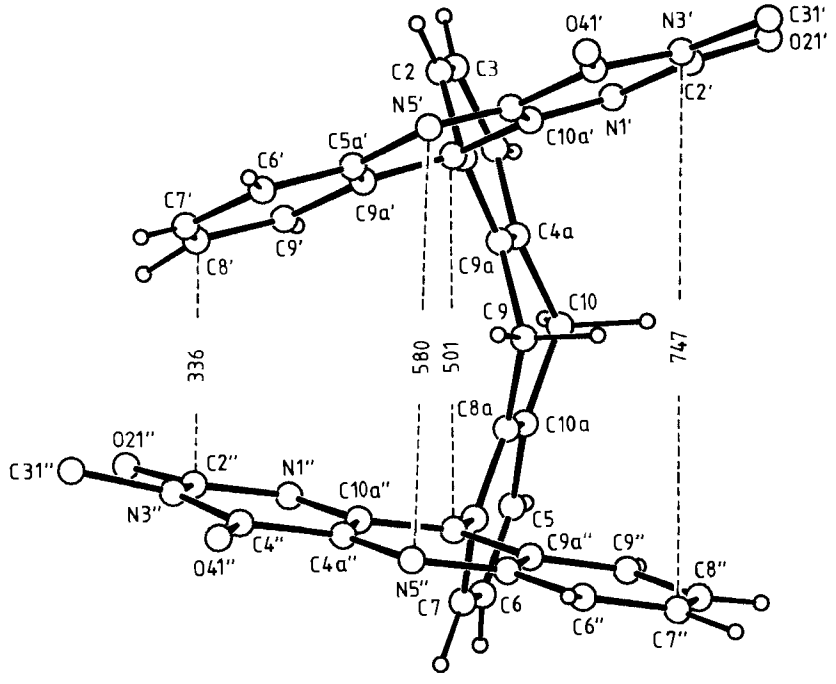


Figure 1. Molecular structure of **26** in a side-view (for a better representation of the structure the molecule is with its C(9)···C(10) axis slightly turned out of the perpendicular orientation to the drawing plane)

rigid, bulky structure which explains the easy accommodation of the solvent molecules in the crystal.

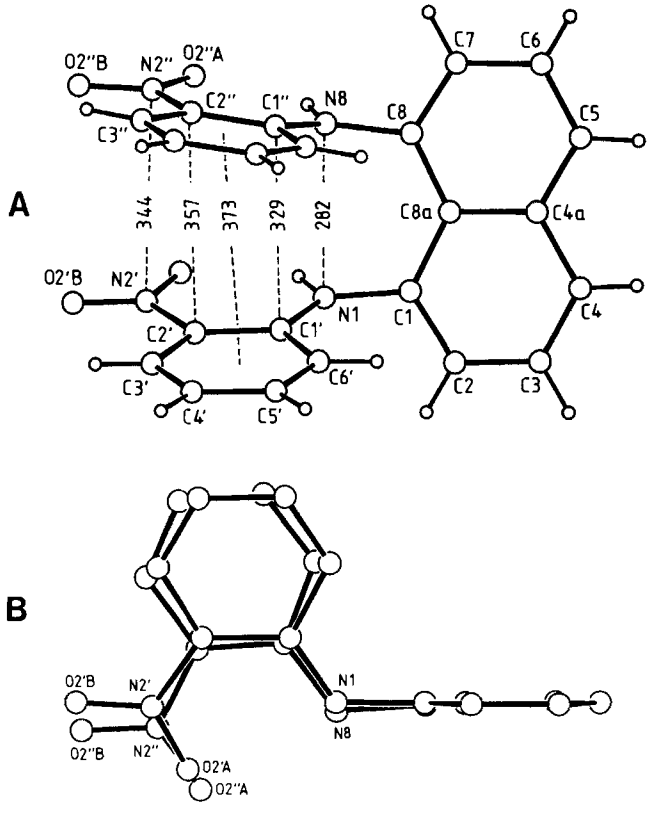


Figure 2. Molecular structure of **22** in a view perpendicular to the naphthalene plane (A) and in a side view along the longer naphthalene axis (B)

1,8-Bis(2'-nitrophenylamino)naphtalene (**22**): Unexpectedly, the two 1,8-substituents of the naphthalene **22** in the crystalline state are oriented to each other in the *syn* conformation which for reasons of steric and polar interactions was considered to be less favored than the *anti* conformation. Figure 2 (A) represents the molecular structure in a view perpendicular to the naphthalene plane; in this figure some intramolecular distances between corresponding atoms of the 1,8-substituents are given. Figure 2 (B) shows **22** in a side-view along the longer axis of the naphthalene unit which demonstrates the nearly ecliptic position of the phenylamino rings and, even more surprising, of the two nitro groups, too. This conformation is obviously the result of an especially favorable crystal packing which is shown in Figure 3. In fact, for solutions of **22** evidence was obtained from ^{1}H-NMR spectra for an equilibrium between the *syn* and *anti* conformations (see above). In contrast to the arene-spacered bis(isoalloxazines), for **22** a rather low barrier for the rotation around the *peri*-bonds C(1)–N and C(8)–N was observed allowing a fast *syn-anti* equilibration.

Experimental

UV/Vis: Cary 2300 (Varian). – IR: Beckmann IR-4240 (KBr), Perkin-Elmer FT-IR 1760 (KBr). – MS: DuPont CEC 21-492; Finnigan MAT 212 (ionization potential 70 eV; only the most prominent peaks are listed, usually with $I_{rel} > 10\%$); FAB spectra (LSI-MS: Liquid Secondary Ion MS, positive; 3-nitrobenzyl alcohol/1% TFA): VG Analytical ZAB 2E/70SE. – ^{1}H NMR: Hitachi Perkin-Elmer R 24B; Bruker Physik HX-360, AM 500 (internal reference tetramethylsilane). – Cyclic voltammetry: Potentiostat PAR 362 (EG & G Princeton Applied Research); "glassy carbon electrode", reference electrode Ag/AgCl (3.5 M KCl/water); 0.1 M tetra-*n*-butylammonium perchlorate (puriss., Fluka) in acetonitrile (purification by azeotropic rectification after addition of 10% v/v methanol, heating with sodium hydride and subsequent distillation, filtration over alumina, rectification over calcium hydride under argon[29]); concentration of measured compounds 10^{-3}–10^{-4} M; voltage increase rate 500 mV · s^{-1}; all measurements performed at 20°C;

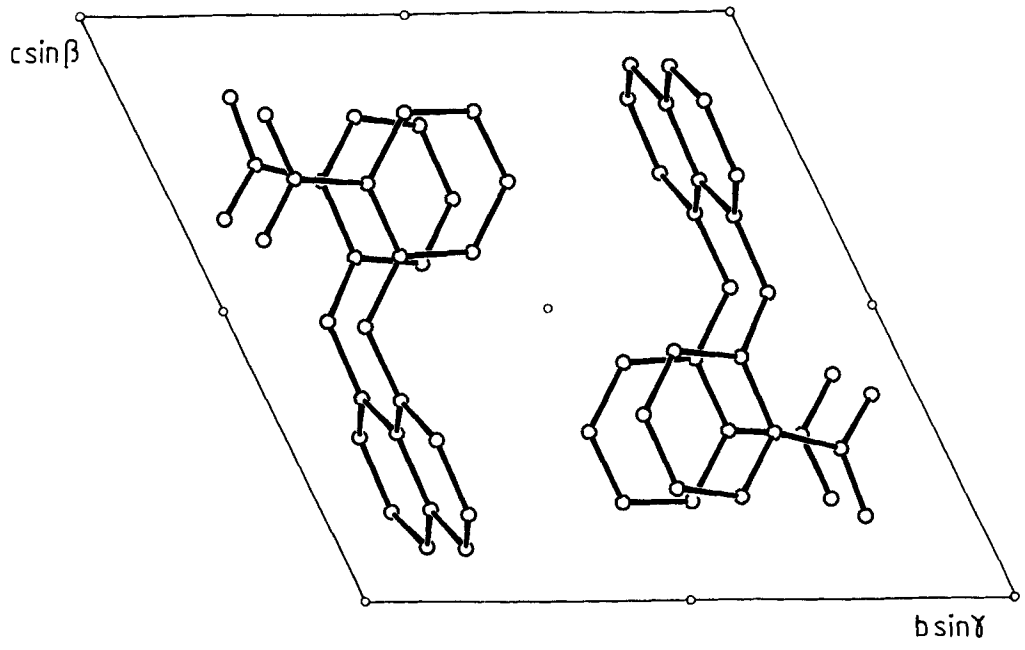


Figure 3. Crystal packing of **22** (shown in a projection along the *a*-axis)

reference ferrocene [E_{ox} (Fc/Fc$^+$) = +0.44 ± 0.01 V]. – Microanalysis: Elemental Analyzer 1106 Carlo Erba. – Analytical TLC: DC Micro Cards Polygram SIL G/UV$_{254}$, Macherey-Nagel. – CC: Silica gel SiliTech 63–200 μm, ICN Biomedicals. – MPLC: Gilson d = 2.8 cm, h = 38 cm; flow rate 30 ml · min^{-1}; detection at λ = 254 nm); silica gel 60, 20–45 μm.

N,N-Trimethylenebis(2-nitroaniline) (**9**): In a modified version of a reported preparation[6a] 16.55 g (105 mmol) of 2-chloronitrobenzene and 3.4 g (50 mmol) of 1,3-diaminopropane in 50 ml of 3-methyl-1-butanol were heated with 7.3 g (52.5 mmol) of potassium carbonate for 24 h at reflux. After cooling, the insoluble product was sucked off, washed with hot petroleum ether (b. p. 40–60°C) and dissolved in hot trichloromethane. After filtration and addition of the double volume of methanol to the solution the product crystallized as yellow needles: 8.22 g (52%, ref.[6a] 39%), m. p. 145–147°C (ref.[6a] 148–149°C).

10,10'-Trimethylenebis(10-isoalloxazine) (**11**): In analogy to ref.[6], a solution of 6.00 g (19.0 mmol) of trimethylene-*N,N'*-bis(2-nitroaniline) and 4.7 g (75.8 mmol) of boric acid in 220 ml of acetic acid was hydrogenated in the presence of 0.70 g of palladium catalyst (10% on charcoal) at 1 bar and 70°C until the end of hydrogen absorption (6 equiv.). The solution was then filtrated under nitrogen from the catalyst into a freshly prepared solution of 7.70 g (48.0 mmol) of alloxan hydrate and 11.9 g (192 mmol) of boric acid in 200 ml of acetic acid. After stirring for 15 h under nitrogen at 60–65°C the precipitated crude product was sucked off and washed subsequently with acetic acid, hot water and ethanol. The precipitate was dissolved in 300 ml of formic acid with addition of 5–10 ml of concd. hydrochloric acid. The dark yellow solution was filtrated, and ca. 300 ml of water was added to the filtrate for the precipitation of the product. After keeping at 2°C for 15 h the product was sucked off and washed with water, methanol and diethyl ether: 3.80 g (43%) of **11** as a yellow crystalline powder, m. p. 280–284°C (dec.); without further purification this product was used for the preparation of **12** and **2**.

10,10'-Trimethylenebis(3-methylisoalloxazine) (**12**): A suspension of 468 mg (1.00 mmol) of **11** in 50 ml of dry DMF was stirred with 5.60 g (40.5 mmol) of pulverized potassium carbonate and 5.68 g (40.0 mmol) of iodomethane for 36 h in the dark at 50°C under nitrogen. The crude product was sucked off, dissolved in 10 ml of formic acid/trichloromethane (9:1), adsorbed on 1 g of Florisil and chromatographed on silica gel (h = 10 cm, d = 5 cm) with trichloromethane/formic acid (4:1). From the bright yellow eluate (R_f ≈ 0.26; silica gel, trichloromethane/methanol/formic acid, 18:1:0.5) the solvents were distilled off in vacuo, and the product was crystallized from 4 ml of hot formic acid by addition of a small amount of water: 350 mg (70%) of **12** as fine yellow needles, m. p. 289–294°C (dec., ref.[6c] m. p. 296°C). – MS: *m/z* (%) = 496 (50, M$^+$), 382 (23), 270 (48), 268 (85), 253 (21), 242 (22), 228 (85), 183 (29), 182 (28), 171 (32), 170 (16), 143 (100), 130 (25), 129 (25), 116 (18), 102 (19). – ^{1}H NMR (360 MHz, [D]TFA, 303 K): δ = 2.87–3.01 (m, 2H, $-CH_2CH_2CH_2-$), 3.76 (s, 6H, 3,3'-CH_3), 5.52 ("t", J = 7 Hz, 4H, $-CH_2CH_2CH_2-$), 8.14–8.18 (m, 2H, 7,7'-H), 8.41 ("d", J ≈ 8.9 Hz, 2H, 9,9'-H), 8.44–8.49 (m, 2H, 8,8'-H), 8.55 ("d", J ≈ 8.3 Hz, 2H, 6,6'-H). For elemental analysis **12** was dried for 20 h at 175°C/6 · 10^{-6} Torr. – $C_{25}H_{20}N_8O_4$ (496.49): Calcd. C 60.48, H 4.06, N 22.57; found C 60.48, H 3.72, N 22.39.

[3.3](3,10)Isoalloxazinophane (**2**): To a suspension of 1.16 g (2.48 mmol) of **11** and 1.37 g (9.93 mmol) of pulverized potassium carbonate in 90 ml of dry DMF at 55–60°C under nitrogen 751 mg (3.72 mmol) of 1,3-dibromopropane was added. Then further 1.512 g (760 μl, 7.49 mmol) of 1,3-dibromopropane was added with stirring within 100 h in 20-μl portions. Subsequently, again 3.98 g (2.00 ml, 19.7 mmol) of 1,3-dibromopropane was added, and the reaction mixture was stirred for additional 2 h. The solvent was distilled off in a rotary evaporator leaving a solid product which was washed with 50 ml each of dichloromethane and trichloromethane. The residue was dissolved in 30 ml of formic acid, filtered, and the filtrate was concentrated in vacuo to half the original volume. To the hot solution water was added until beginning turbidity. On cooling for 12 h to 2°C the precipitate formed was sucked off and washed with water, methanol, dichloromethane, and diethyl ether to afford 315 mg (yield 25%) of crude **2** as greenish-yellow crystal powder; 528 mg of this product **2** (combined from two reactions) was dissolved in 4 ml of hot formic acid and purified by CC (h = 20, d = 5 cm; silica gel; trichloromethane/formic acid/methanol from 9:1:1 to 8:2:0.5). The product fractions were combined according to TLC (R_f ≈ 0.23; silica gel; trichloromethane/formic acid/methanol, 18:1:2) from which no indication for the presence of the second isomer was obtained. Crystallization from formic acid by addition of water furnished 246 mg (yield 47%, related to the product before chromatographic purification) of fine yellow needles, m. p. 348–350°C (dec., under argon). – MS: *m/z* (%) = 508 (28, M$^+$), 256 (28), 255 (54), 254 (100), 215 (30), 211 (22), 171 (42), 170 (66). – ^{1}H NMR (360 MHz, [D]TFA, assignment by NOE and comparison with reference compounds): δ = 2.67–2.80 (m, 2H, 14,14'-H), 3.37–3.50 (m, 2H, 12,12'-H), 4.37–4.41 (m, 2H, 13,13'-H), 4.68–4.72 (m, 2H, 11,11'-H), 4.85–4.89 (m, 2H, 13,13'-H), 5.33–5.40 (m, 2H, 11,11'-H), 8.12–8.16 (m, 2H, 7,7'-H), 8.21 ("d", J ≈ 8.6 Hz, 2H, 9,9'-H), 8.37–8.41 (m, 2H, 8,8'-H), 8.59 ("d", J ≈ 7.6 Hz, 2H, 6,6'-H). The ^{1}H-NMR data indicated that of the two possible reaction products only the chiral isomer **2** was obtained the presence of which was confirmed by ^{1}H NMR with a chiral cosolvent (see above). For elemental analysis **2** was dried at 120°C/10^{-3} Torr; nevertheless, the analysis indicated the presence of a monohydrate of **2**. – $C_{26}H_{20}N_8O_4 \cdot H_2O$ (526.50): Calcd. C 59.31, H 4.21, N 21.28; found C 59.34, H 4.51, N 21.51. – $C_{26}H_{20}N_8O_4$: Calcd. 508.1607, found 508.1604 (MS:M$^+$).

1,3-Bis(3'-nitrophenyl)-2-propen-1-one: Preparation according to ref.[16] from 15.1 g (0.10 mol) of 3-nitrobenzaldehyde and 16.5 g (0.10 mol) of 3-nitroacetophenone yielded 12.0 g of the corresponding chalcone (41%; m. p. 206°C, from acetic acid). – MS: *m/z* (%) = 298 (80, M$^+$), 297 (40), 281

(100), 253 (25), 252 (40), 176 (85), 164 (50). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 7.75–7.77 (m, 1H, 5′-H), 7.88–7.90 (m, 1H, 5-H), 7.93 (d, *J* = 15.5 Hz, 1H, –C*H*=CH-CO–), 8.22 (d, *J* = 15.5 Hz, 1H, –CH=C*H*-CO–), 8.27–8.30 (m, 1H, 6′-H), 8.36–8.39 (m, 1H, 6-H), 8.50–8.53 (m, 1H, 4′-H), 8.63–8.66 (m, 1H, 4-H), 8.79–8.81 (m, 1H, 2′-H), 8.85–8.87 (m, 1H, 2-H). – $C_{15}H_{10}N_2O_5$ (298.25): Calcd. C 60.41, H 3.38, N 9.39; found C 60.50, H 3.48, N 9.22.

1,3-Bis(3-acetylaminophenyl)-1-propanol: 10.3 g (34 mmol) of the aforementioned dinitrochalcone in 200 ml of methanol in the presence of 1.2 g of palladium catalyst (10% on charcoal) and of 100 mg of 4-toluenesulfonic acid was hydrogenated at 20°C for 18 h at 4 bar. The filtered solution after addition of 20.0 ml of acetic anhydride was refluxed for 2 h. After cooling the solution was poured into 700 ml of ice water, and after 1 h the crystalline precipitate was filtered off: 10.6 g (yield 97%), m. p. 152–153°C (from toluene). – MS: *m/z* (%) = 326 (9, M$^+$), 178 (30), 164 (20), 163 (40), 162 (17), 149 (100). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 1.82–1.99 (m, 2H, –C*H*$_2$CH$_2$CH(OH)–), 2.02 (s, 6H, COCH$_3$), 2.54–2.56 [m, 2H, –CH$_2$-C*H*$_2$-CH(OH)–], 4.47–4.52 (m, 1H, –C*H*(OH)–), 5.08 (d, *J* = 4.4 Hz, 1H, OH), 6.84 (d, *J* = 7.6 Hz, 1H, 4′-H), 6.99 (d, *J* = 7.6 Hz, 1H, 4-H), 7.15 ("t", *J* ≈ 7.6 Hz, 1H, 5′-H), 7.21 ("t", *J* ≈ 7.8 Hz, 1H, 5-H), 7.37 (d, *J* = 7.6 Hz, 1H, 6′-H), 7.38 (s, 1H, ar-2′-H), 7.47 (d, *J* = 8.0 Hz, 1H, 6-H), 7.51 (s, 1H, 2-H), 9.66 (s, 1H, NH), 9.72 (s, 1H, NH). – $C_{19}H_{22}N_2O_3$ (326.38): Calcd. C 69.92, H 6.79, N 8.58; found C 69.96, H 6.86, N 8.69.

1,1′-Trimethylenebis(3-acetylaminobenzene) (**13**): Of the product obtained in the preceding reaction 10.0 g (31 mmol) in 250 ml of methanol in the presence of 1.5 g of palladium catalyst (10% on charcoal) and of 120 mg of 4-toluenesulfonic acid was hydrogenated at 4 bar (20°C, 24 h). After filtration of the catalyst, the solution was concentrated to about 125 ml by evaporation of the solvent. **13** crystallized as colorless rhombs: 9.0 (yield 95%), m. p. 150–153°C. – MS: *m/z* (%) = 310 (40, M$^+$), 162 (46), 149 (58), 120 (12), 107 (100). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 1.81–1.85 (quint, *J* = 7.6 Hz, 2H, –CH$_2$-C*H*$_2$-CH$_2$–), 2.02 (s, 6H, CO-CH$_3$), 2.55 (t, *J* = 7.6 Hz, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 6.87 (d, *J* = 7.6 Hz, 2H, 4,4′-H), 7.18 ("t", *J* ≈ 7.7 Hz, 2H, 5,5′-H), 7.39 (d, *J* = 7.7 Hz, 2H, 6,6′-H), 7.41 (s, 2H, 2,2′-H), 9.81 (br. s, 2H, NH). – $C_{19}H_{22}N_2O_2$ (310.38): Calcd. C 73.52, H 7.14, N 9.03; found C 73.48, H 7.38, N 8.92.

1,1′-Trimethylenebis[3-(acetylmethylamino)benzene] (**14**): A mixture of 31.0 g (0.1 mol) of **13** and of 22.0 ml (0.35 mol) of iodomethane in 200 ml of dichloromethane together with 68.0 g (0.2 mol) of tetra-*n*-butylammonium hydrogen sulfate in 200 ml of a 2 N aqueous solution of sodium hydroxide were heated to boiling for 3 h. After cooling the dichloromethane phase was separated, concentrated in vacuo and the residue was diluted with 200 ml of diethyl ether. The precipitate formed was sucked off and washed with diethyl ether; a second fraction of the product was obtained by evaporating the solvent from the filtrate and letting the resulting yellow oil crystallize. Thus, 30.0 g (yield 89%) of **14** as small colorless needles, m. p. 71–73°C (from ethyl acetate/petroleum ether, b. p. 60°C) were obtained. – MS: *m/z* (%) = 338 (75, M$^+$), 296 (53), 295 (35), 253 (16), 176 (35), 163 (25), 121 (100). – ^{1}H NMR (360 MHz, CDCl$_3$): δ = 1.87 (s, 6H, CH$_3$CO), 1.96 ("quint", *J* ≈ 7.7 Hz, 2H, –CH$_2$-C*H*$_2$-CH$_2$–), 2.69 (t, *J* = 7.7 Hz, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 3.26 (s, 6H, N-CH$_3$), 7.01 (s, 2H, 2,2′-H), 7.02 ("d", *J* ≈ 7.7 Hz, 2H, 6,6′-H), 7.15 (d, *J* = 7.6 Hz, 2H, 4,4′-H), 7.33 ("t", *J* ≈ 7.7 Hz, 2H, 5,5′-H). – $C_{21}H_{26}N_2O_2$ (338.45): Calcd. C 74.53, H 7.74, N 8.28; found C 74.44, H 7.57, N 8.07.

1,1′-Trimethylenebis[3-(methylamino)benzene] (**15**): 33.8 g (0.1 mol) of **14**, 700 ml of ethanol and a solution of 507.0 g (9.0 mol) of potassium hydroxide in 350 ml of water were heated under reflux for 4 h. After cooling the reaction mixture was decanted from potassium hydroxide and neutralized with ice cooling with ca. 200 ml of concd. hydrochloric acid. The precipitate formed was sucked off and washed with dichlormethane; the filtrate was extracted with dichloromethane. The combined dichloromethane solutions were washed with water and a saturated sodium chloride solution, then dried with anhydrous magnesium sulfate. The solvent was distilled off, and the oily residue purified by CC on silica gel with dichloromethane to afford 23.6 g of **15** (yield 93%), R_f ≈ 53 (silica gel, dichloromethane/acetone, 1:1). – MS: *m/z* (%) = 254 (62, M$^+$), 240 (11), 134 (16), 121 (100). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 1.81 (quint, *J* = 7.6, 2H, –CH$_2$–C*H*$_2$-CH$_2$–), 2.47 (t, *J* = 7.6 Hz, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 2.64 (d, *J* = 3.4 Hz, 6H, N-CH$_3$), 5.42 (br. s, 2H, NH), 6.33–6.38 (m, 6H, 2,2′, 4,4′, 6,6′-H), 6.97 ("t", *J* ≈ 7.6 Hz, 2H, 5,5′-H). – $C_{17}H_{22}N_2$ (254.37): Calcd. C 80.27, H 8.72, N 11.01; found C 80.13, H 8.78, N 11.11.

1,1′-Trimethylenebis{3-[methyl(6-uracilyl)amino]benzene} (**16**): 500 mg (2 mmol) of **15** and 500 mg (3.4 mmol) of 6-chlorouracil were heated to 200°C until an orange-yellow foam had formed (ca. 15 min). After cooling the crude product was dissolved in 20 ml of hot ethanol and chromatographed on silica gel (trichloromethane/methanol, 9:1, R_f ≈ 0.28, blue fluorescence): 597 mg (yield 64%) of **16**, m. p. 258–260°C (from ethanol). – MS: *m/z* (%) = 474 (45, M$^+$), 431 (15), 365 (15), 364 (60), 320 (38), 244 (65), 231 (34), 121 (100), 120 (29). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 1.91 (quint, *J* = 7.5 Hz, 2H, –CH$_2$–C*H*$_2$-CH$_2$–), 2.63 (t, *J* = 7.5 Hz, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 3.23 (s, 6H, N-CH$_3$), 4.26 (s, 2H, urac-5-H), 7.07 (d, *J* = 7.1 Hz, 2H, 6,6′-H), 7.11 (s, 2H, 2,2′-H), 7.16 (d, *J* = 7.7 Hz, 2H, 4,4′-H), 7.35 ("t", *J* ≈ 7.7 Hz, 2H, 5,5′-H), 10.14 (s, 2H, NH), 10.35 (s, 2H, NH). – $C_{25}H_{26}N_6O_4$ (474.51): Calcd. C 63.28, H 5.52, N 17.71; found C 63.21, H 5.58, N 17.48.

1,1′-Trimethylenebis[8-(10-methylisoalloxazine 5-oxide)] (**17**): To a solution of 6.0 g (12.6 mmol) of **16** in 50 ml of glacial acetic acid 9.1 g (130 mmol) of sodium nitrite was added. The mixture was stirred for 4 h at room temp.; then 500 ml of water was added. After another 12 h the orange precipitate was sucked off, washed with water, methanol and diethyl ether. By recrystallization from formic acid **17** (3.2 g; yield 48%) was obtained as orange needles, dec. > 276°C; R_f ≈ 0.13 (silica gel; trichloromethane/methanol, 9:1). – $C_{25}H_{20}N_8O_6$ (528.48): Calcd. C 56.82, H 3.81, N 21.20; found C 56.88, H 3.71, N 20.91.

1,1′-Trimethylenebis[8-(10-methylisoalloxazine)] (**18**): To 3.2 g (6.1 mmol) of **17** a solution of 6.3 g (40 mmol) of sodium dithionite in 65 ml of water was added, and the reaction mixture was stirred at room temp. for 6 h; then 1.2 ml of a 30% aqueous solution of hydrogen peroxide was added. After standing for 12 h precipitate was sucked off, washed with water and acetone, and recrystallized from 100 ml of formic acid by addition of a small amount of water: 1.24 g (yield 41%) of **18**, yellow crystal powder, m. p. 328–330°C. – MS: *m/z* (%) = 496 (18, M$^+$), 482 (25), 256 (28), 254 (30), 242 (100), 228 (28), 213 (14), 211 (15), 199 (30), 184 (13), 171 (19), 170 (18), 169 (14), 157 (27), 156 (17), 144 (13). – ^{1}H NMR (500 MHz, [D]TFA): δ = 2.37 (quint, *J* = 8.1 Hz, 2H, –CH$_2$-C*H*$_2$-CH$_2$–), 3.37 (t, *J* = 8.1, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 4.68 (s, 6H, N-CH$_3$), 8.20 (dd, *J* = 8.7 and 1.2 Hz, 2H, 7,7′-H), 8.33 (s, 2H, 9,9′-H), 8.58 (d, *J* = 8.7 Hz, 2H, 6,6′-H). – $C_{25}H_{20}N_8O_4$ (496.47): Calcd. C 60.48, H 4.06, N 22.57; found C 60.27, H 3.98, N 22.60.

1,1′-Trimethylenebis[8-(3,10-dimethylisoalloxazine)] (**19**): 496 mg (1.0 mmol) of **18** in 50 ml of dried DMF was stirred for 6 h under argon at 50°C with 5.6 g (40 mmol) of pulverized potassium carbonate and 5.7 g (40 mmol) of iodomethane. After addition of 50 ml of water the reaction mixture was stirred for further 18 h at room temp. The yellow precipitate was sucked off, washed with water and acetone, and crystallized from glacial acetic acid: 123 mg (yield 24%) of **19** as fine yellow needles, m. p. 324°C. – MS: *m/z* (%) = 524 (8, M$^+$), 510 (24), 496 (22), 270 (30), 256 (80), 242 (100), 213 (30), 199 (33), 185 (30), 171 (25), 170 (25), 156 (30). – ^{1}H NMR (500 MHz, [D]TFA): δ = 2.41 (quint, *J* = 8.0 Hz, 2H, –CH$_2$-C*H*$_2$-CH$_2$–), 3.37 (t, *J* = 8.0 Hz, 4H, –C*H*$_2$-CH$_2$-C*H*$_2$–), 3.77 (s, 6H, 3.3′-CH$_3$), 4.67 (s, 6H, 10,10′-CH$_3$), 8.19 (dd, *J* = 8.7 and 1.2 Hz, 2H, 7,7′-H), 8.32 (s, 2H, 9,9′-H), 8.57 (d, *J* = 8.7 Hz, 2H, 6,6′-H). – $C_{27}H_{24}N_8O_4$ (524.52): Calcd. C 61.83, H 4.61, N 21.36; found C 61.97, H 4.90, N 21.30.

10,10′-Dimethyl[3.3](3,8)isoalloxazinophane (**4**): To a stirred suspension of 744 mg (1.5 mmol) of **18** and 830 mg (6.0 mmol) of pulverized potassium carbonate in 75 ml of dried DMF was added at 55–60°C under argon 172 µl (1.5 mmol) of 1,3-diiodopropane, within additional 96 h 0.6 ml (5.25 mmol) of 1,3-diiodopropane in four portions and towards the end of the reaction time further 1.4 ml (12 mmol) of 1,3-diiodopropane were added. After stirring of the reaction mixture (24 h, room temp.) the solvent was removed in vacuo, and the dark green residue was washed twice with 100 ml each of trichloromethane and acetone. The crude product obtained from two such reactions with 5 ml of formic acid was subjected to CC (*h* = 20 cm, *d* = 5 cm; silica gel; trichloromethane/methanol, 9:1); the product which remained undissolved in the starting zone was extracted with formic acid and chromatographed again. The product fractions (TLC: silica gel, trichloromethane/methanol, 9:1; R_f ≈ 0.16, orange fluorescence) were combined, and by concentration in vacuo 30 mg (yield 3.7%) of **4** precipitated as orange microcrystals, m. p. 368–370°C (dec.). – MS: *m/z* (%) = 536 (100, M$^+$), 522 (95), 508 (55), 364 (25), 328 (30), 282 (45), 268, (40), 256 (30), 243 (38), 242 (30), 228 (55), 212 (30), 211 (25), 199 (30), 198 (35), 196 (32), 185 (45), 184 (43), 171 (35), 170 (35), 157 (40), 156 (57), 151 (25), 144 (28), 143 (45), 131 (25), 117 (25), 104 (25), 56 (90). – ^{1}H NMR (500 MHz, [D]TFA; assignment by COSY and ROE, see above): δ = 2.68–2.75 (m, 2H, 12,12′-H), 2.81–2.88 (m, 2H, 14,14′-H), 3.29–3.41 (m, 4H, 11,11′-H), 4.48 (s, 6H, 10,10′-CH$_3$), 4.50–4.56 (m, 2H, 13,13′-H), 4.65–4.71 (m, 2H, 13,13′-H), 7.66 (s, 2H, 9,9′-H), 8.22 (d, *J* = 8.6 Hz, 2H, 7,7′-H), 8.30 (d, *J* = 8.6 Hz, 2H, 6,6′-H). – In the conventional elemental analyses the data obtained were not correct within the normal limits (inclusion of solvent?); high-resolution MS, however, confirmed the formation of **4**. – $C_{28}H_{24}N_8O_4$ (536.55): Calcd. C 62.68, H 4.51, N 20.88; found C 61.15, H 4.24, N 20.53; calcd. 536.1920 (M$^+$), found 536.1936 (MS).

1,1′-Trimethylenebis[3-(10-methylisoalloxazine)] (**20**): A suspension of 456 mg (2.0 mmol) of 10-methylisoalloxazine[18] and 1.1 g (8.0 mmol) of pulverized potassium carbonate in 70 ml of dry DMF was stirred for 3 h at

55–60°C under argon after addition of 114 µl (1.0 mmol) of 1,3-diiodopropane. Then further 228 µl (2.0 mmol) of 1,3-diiodopropane was added and the reaction mixture was stirred again at 55–60°C for two more days. After filtration and evaporation of the solvent from the filtrate, to the residue 100 ml each of trichloromethane and water were added. The aqueous phase was extracted twice with 50 ml of trichloromethane each, and the combined organic phases were washed with a 0.1 M of sodium hydroxide solution, 0.1 M of hydrochloric acid and water. After drying with anhydrous magnesium sulfate the solvent was distilled off in vacuo, and the raw product, adsorbed on Florisil from formic acid, was chromatographed on silica gel with trichloromethane/methanol (9:1) as the eluent. The eluate ($R_f \approx 0.38$) crystallized from formic acid by addition of a small amount of water to furnish orange crystals, m. p. 304°C; 120 mg (yield 24%). – MS: *m/z* (%) = 496 (10, M^+), 482 (18), 268 (98), 254 (100), 242 (20), 241 (30), 229 (40), 214 (40), 185 (45), 180 (45), 170 (62), 143 (72), 135 (72). – ^{1}H NMR (500 MHz, [D]TFA): δ = 2.53 (quint, *J* = 7.0 Hz, 2H, $-CH_2-CH_2-CH_2-$), 4.52 (t, *J* = 7.0 Hz, 4H, $-CH_2-CH_2-CH_2-$), 4.68 (s, 6H, 10,10′-CH_3), 8.28 ("t", $J \approx 7.7$ Hz, 2H, 7,7′-H), 8.42 ("d", $J \approx 8.8$ Hz, 2H, 9,9′-H), 8.53 ("dt", *J* = 7.4 and 1.3 Hz, 2H, 8,8′-H), 8.63 ("dd", $J \approx 8.4$ and 1.0 Hz, 2H, 6,6′-H). – $C_{25}H_{20}N_8O_4$ (496.48): Calcd. C 60.48, H 4.06, N 22.57; found C 60.53, H 4.25, N 22.36.

1,1′-Trimethylenebis[3-(8,10-dimethylisoalloxazine)] (**21**): In analogy to the preparation of **20**, 242 mg (1.0 mmol) of 8,10-dimethylisoalloxazine[17] was treated with 1,3-diiodopropane in DMF in the presence of potassium carbonate. The product obtained by working up the reaction mixture as for **20** was chromatographed on silica gel with trichloromethane/methanol (9:1) as the eluent, $R_f \approx 0.61$; 9.0 mg (yield 3.4%) of orange crystals, m. p. 300°C (dec.). – MS: *m/z* (%) = 524 (5, M^+), 510 (11), 282 (22), 269 (19), 268 (100), 243 (13), 242 (11), 229 (18), 185 (17), 184 (24). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.23 (quint, *J* = 6.9 Hz, 2H, $-CH_2-CH_2-CH_2-$), 2.66 (s, 6H, 8,8′-CH_3), 4.11 (s, 6H, 10,10′-CH_3), 4.27 (t, *J* = 6.9 Hz, 4H, $-CH_2-CH_2-CH_2-$), 7.42 (s, 2H, 9,9′-H), 7.43 (d, *J* = 8.3 Hz, 2H, 7,7′-H), 8.16 (d, *J* = 8.3 Hz, 2H, 6,6′-H). – $C_{27}H_{24}N_8O_4$: Calcd. 524.1920 (M^+), found 524.1903 (MS).

1,8-Bis(2′-nitrophenylamino)naphthalene (**22**): To a vigorously stirred solution of 10.0 g (63.2 mmol) of 1,8-diaminonaphthalene in 150 ml of dry DMSO was added under argon 41.6 g (632 mmol) of potassium hydroxide (85% KOH). Then, with ice cooling, 33.4 ml (316 mmol) of 2-fluoronitrobenzene was added dropwise at temperatures between 15 and 20°C. After 20 min the temperature was raised to 45–50°C for 4 h. The reaction mixture was poured on 700 g of crushed ice, acidified with 70 ml of concd. hydrochloric acid, and extracted six times with 100 ml each of dichloromethane. The combined organic phases were dried with anhydrous magnesium sulfate, then the solvent was evaporated in vacuo. CC (*h* = 22 cm, *d* = 10 cm; silica gel, toluene; $R_f \approx 0.35$) and subsequent crystallization from toluene yielded 1.34 g (yield 5.3%) of **22**, ruby needles, m.p. 207–209°C. – MS: *m/z* (%) = 400 (100, M^+), 320 (14), 319 (13), 308 (16). – ^{1}H NMR (500 MHz, CD_2Cl_2, COSY assignment): δ = 6.59–6.61 (m, 2H, 4′-H), 6.66 (d, *J* = 8.1 Hz, 2H, 6′-H), 7.11–7.13 (m, 2H, 5′-H), 7.45 (d, *J* = 7.6 Hz, 2H, 2,7-H), 7.53–7.56 (m, 2H, 3,6-H), 7.92 (d, *J* = 8.4 Hz, 2H, 4,5-H), 7.95 (dd, *J* = 8.5 and 1.5 Hz, 2H, 3′-H), 9.62 (br. s, 2H, NH). – X-Ray structure analysis of **22** see above. – $C_{22}H_{16}N_4O_4$ (400.38): Calcd. C 65.99, H 4.03, N 13.99; found C 65.81, H 4.02, N 13.87.

Structure of 1,8-Bis(2′-nitrophenylamino)naphthalene (**22**): **22** crystallized from toluene as ruby needles (0.25 × 0.10 × 0.1 mm); $C_{22}H_{16}N_4O_4$; molecular mass: 400.4; triclinic space group *P*1 (No. 2, Intern. Tables); cell parameters: *a* = 822.5(1), *b* = 1134.1(2), *c* = 1153.1(2) pm; α = 109.94(2)°, β = 106.44(2)°, γ = 102.47(2)°; *Z* = 2, D_x = 1.462 g · cm^{-3}; measurements (details see above) resulted in 3550 symmetry-independent reflections of which 2665 with $I \geq 3.0\ \sigma(I)$ were classified as observed. The structure was solved by direct method (SIR); the refinement including all non-hydrogen atoms with anisotropic and the hydrogen atoms with isotropic temperature factors resulted in *R* = 0.034. For atomic coordinates and equivalent isotropic thermal parameters for the non-hydrogen atoms see ref.[28].

anti-1,8-Bis(3′-methylisoalloxazin-10′-yl)naphthalene (**6**): 400 mg (1.0 mmol) of **22** in 60 ml acetic acid was hydrogenated (100 mg of palladium catalyst, 5% on charcoal; room temp.). The resulting solution of the diamine **23** was filtered under argon into a stirred suspension of 640 mg (4.0 mmol) of alloxan hydrate and 2.0 g (32.4 mmol) of boric acid in 60 ml of acetic acid. After 4 h at room temp. the solvent was evaporated in vacuo, and boric acid was removed by fivefold distillation with 100 ml each of methanol/acetic acid (10:1). To the dispersion of the solid residue in 150 ml of dry DMF 2.76 g (20.0 mmol) of potassium carbonate and 1.25 ml (20.0 mmol) of iodomethane were added, and the mixture was stirred at 20°C for 18 h. After evaporation of the solvent the crude product was dissolved in trichloromethane/methanol (10:1) and the solution was filtrated through a 5-cm layer of silica gel and purified by CC (*h* = 20 cm, *d* = 5 cm; silica gel, trichloromethane/methanol, 10:1; $R_f \approx 0.41$): 40.6 mg of **6** (yield 7%, related to **22**), orange crystal powder, m. p. > 400°C (dec.). – MS (LSI-MS): *m/z* = 583, 582, 581 (MH^+), 580 (M^+). – ^{1}H NMR (500 MHz, [D_6]DMSO; assignment by COSY): δ = 2.95 (s, 6H, 3′-CH_3), 6.81 (d, *J* = 8.2 Hz, 2H, 9′-H), 7.50–7.52 (m, 2H, 7′-H), 7.54–7.56 (m, 2H, 8′-H), 7.66 (d, *J* = 7.1 Hz, 2H, 2,7-H), 7.95 (dd, *J* = 7.9 and 1.5 Hz, 2H, 6′-H), 7.97–7.99 (m, 2H, 3,6-H), 8.59 (d, *J* = 8.2 Hz, 2H, 4,5-H). – $C_{32}H_{20}N_8O_4$ (580.54): Calcd. C 66.20, H 3.47, N 19.30; found C 66.22, H 3.42, N 19.08.

1,8-Diamino-9-anthrone: To a vigorously stirred suspension of 21.4 g (90 mmol) of diaminoanthraquinone[22] in a mixture of 1 l of aqueous ammonia (25%) and 100 ml of isopropyl alcohol at 90°C under argon 80.0 g (1.22 mol) of zinc dust was added in small portions during 1 h. Then the temperature was raised to 120°C for another 1 h. After cooling, the reaction mixture was extracted six times with 100 ml each of diethyl ether. The combined ethereal extracts were dried with anhydrous magnesium sulfate, the solvent was evaporated, and the residue was crystallized from toluene: 15.0 g (74%) of 1,8-diamino-9-anthrone as orange crystals, m. p. 129–130°C. – MS: *m/z* (%) = 224 (100, M^+), 223 (16). – ^{1}H NMR (500 MHz, [D_6]DMSO; assignment by NOE 10-CH_2/4,5-H): δ = 4.15 (s, 2H, 10-CH_2), 6.48 (d, *J* = 7.2 Hz, 2H, 4,5-H), 6.61 (d, *J* = 8.3 Hz, 2H, 2,7-H), 7.15–7.18 (m, 2H, 3,5-H), 7.38–7.42 (br. m, 4H, NH_2). – $C_{14}H_{12}N_2O$ (224.25): Calcd. C 74.99, H 5.38, N 12.49; found C 74.88, H 5.33, N 12.35.

1,8-Diaminoanthracene: A mixture of 10.0 g (44.6 mmol) of 1,8-diamino-9-anthrone, 6.78 (178 mmol) of sodium borohydride and 10 ml of dry 1,4-dioxane was heated under argon to 140°C for 18 h. After cooling the brown solid was dissolved in 100 ml of methanol, the solution was poured in 500 ml of water and extracted four times with 50 ml each of ethyl acetate. The combined extracts were washed with water, filtered over alumina (3-cm layer), and the solvent was evaporated to afford 8.6 g (92%) of crude 1,8-diaminoanthracene sufficiently pure for the following reaction. For characterization the product was purified by sublimation (140°C/1 Torr) or by reprecipitation from ethyl acetate/petroleum ether (b. p. 40°C). Crystallization from ethyl acetate/petroleum ether (p. p. 40°C) furnished air-sensitive, bright yellow needles, m. p. 149–151°C (ref.[21] 154–156°C). – MS: *m/z* (%) = 208 (100, M^+), 180 (30), 104 (14). – ^{1}H NMR (500 MHz, [D_6]DMSO, assignment by NOE): δ = 5.83 (br. s, 4H, NH_2), 6.53–6.56 (m, 2H, 3,6-H), 7.16–7.20 (m, 4H, 2,4,5,7-H), 8.16 (s, 1H, 10-H), 8.79 (s, 1H, 9-H). – $C_{14}H_{12}N_2$ (208.25): Calcd. C 80.74, H 5.81, N 13.45; found C 80.65, H 5.99, N 13.31.

1,8-Bis(2′-nitrophenylamino)anthracene (**24**): To a mixture of 9.61 g (46.1 mmol) of 1,8-diaminoanthracene and 30.0 g (535 mmol) of potassium hydroxide powder (85% KOH) in 200 ml of dry DMSO 14.6 ml (138 mmol) of 2-fluoronitrobenzene was added dropwise under argon with cooling and vigorous stirring so that the reaction temperature did not exceed 30°C. After 2.5 h at 20°C the reaction mixture was poured on 400 g of crushed ice and acidified by addition of 50 ml of concd. hydrochloric acid. The precipitate formed was sucked off after 1 h and washed with water, ethanol, and diethyl ether. The brown solid was then extracted with 300 ml of toluene for 3 h in a Soxhlet extractor. The hot extract was decanted onto a silica gel CC (*h* = 16 cm, *d* = 8 cm) and chromatographed with toluene. The residue from the decantation was three times treated with hot toluene, decanted onto a column and chromatographed as described. The fractions of $R_f \approx 0.25$ yielded 565 mg (2.7%) of **24** as bright red platelets, m. p. 290°C (dec., from toluene). – MS: *m/z* (%) = 450 (100, M^+), 370 (13), 369 (11). – ^{1}H NMR (500 MHz, [D_6]DMSO; assignment by COSY and NOE): δ = 6.46 (d, *J* = 8.2 Hz, 2H, 6′-H), 6.68–6.70 (m, 2H, 4′-H), 7.14–7.16 (m, 2H, 5′-H), 7.54 (d, *J* = 7.1 Hz, 2H, 2,7-H), 7.61–7.64 (m, 2H, 3,6-H), 8.03 (d, *J* = 8.7 Hz, 2H, 3′-H), 8.13 (d, *J* = 8.4 Hz, 2H, 4,5-H), 8.41 (s, 1H, 9-H), 8.82 (s, 1H, 10-H), 9.56 (br. s, 2H, NH). – $C_{26}H_{18}N_4O_4$ (450.43): Calcd. C 69.32, H 4.03, N 12.44; found C 69.38, H 4.00, N 12.21.

anti-1,8-Bis(3′-methylisoalloxazin-10′-yl)anthracene (**8**) *and anti-1,8-Bis(3′-methylisoalloxazin-10′-yl)-9,10-dihydroanthracene* (**26**): A suspension of 200 mg (0.44 mmol) of **24** in 70 ml of DMF was hydrogenated in the presence of 1.1 g of palladium catalyst (5% on charcoal) until the starting material had completely dissolved and the color of the solution had changed from orange to greenish-yellow (3 h, room temp.). Under argon the solution was filtered into 300 ml of degassed water, and the aqueous phase was extracted three times with 30 ml each of diethyl ether. From the combined extracts the solvent was evaporated and the residue was dissolved in 50 ml of acetic acid. The resulting solution of **25** was added under argon to a suspension of 211 mg (1.32 mmol) of alloxan hydrate and 2.0 g (32.4 mmol) of boric acid in 50 ml of acetic acid. After stirring for 4 h (room temp., under argon) the solvent was evaporated under reduced pressure. The boric acid was removed by fivefold distillation with 100 ml each of methanol/acetic acid (10:1) from the residue which was dried in vacuo. The resulting brown solid and 1.21 g (8.8 mmol) of potassium carbonate were dispersed in a mixture of 100 ml of dry DMF and 0.55 ml (8.8 mmol) of iodomethane and the mixture was stirred for 18 h at room temp. under argon. The solvent was removed at reduced pressure, the residue was dissolved in trichloromethane/

methanol (10:1) and the solution filtered through silica gel (9-cm layer). By subsequent MPLC separation (silica gel, trichloromethane/methanol, 40:1, other parameters see above) three isoalloxazine-containing fractions (**8**: $R_f \approx 0.12$; **26**: $R_f \approx 0.17$; **27**: $R_f \approx 0.42$) were obtained.

8: 23.8 mg (8.6%), orange platelets of m. p. 315°C (dec., from acetonitrile). – MS (LSI-MS): *m/z* = 633, 632, 631 (MH^+), 630 (M^+). – ^{1}H NMR (500 MHz, [D$_6$]DMSO; assignment by COSY): δ = 3.16 (s, 6H, 3′-CH$_3$), 6.58 (d, *J* = 9.1 Hz, 2H, 9′-H), 6.65 (s, 1H, 9-H), 7.47–7.49 (m, 2H, 7′-H), 7.51–7.53 (m$_c$, 2H, 8′-H), 7.71 (d, *J* = 7.1 Hz, 2H, 2,7-H), 7.84–7.86 (m$_c$, 2H, 3,6-H), 8.08 (d, *J* = 7.7 Hz, 2H, 6′-H), 8.53 (d, *J* = 8.6 Hz, 2H, 4,5-H), 9.18 (s, 1H, 10-H); for ^{1}H-NMR analysis in the presence of the chiral shift reagent Eu(TFC)$_3$[20] see above. – $C_{36}H_{22}N_8O_4$: Calcd. 630.1764 (M^+), found 630.1794 (MS); Calcd. 631.1842 (MH^+), found 631.1854 (MS).

26: 13.4 mg (4.8%), orange microcrystals of m. p. 310°C (dec., from dichloromethane). – MS (LSI-MS): *m/z* = 636, 635, 634, 633 (MH^+), 632 (M^+). – ^{1}H NMR (500 MHz, CD_2Cl_2; assignment by COSY): δ = 3.02 (s, 2H, 9-CH$_2$), 3.29 (s, 6H, 3′-CH$_3$), 4.39 (s, 2H, 10-CH$_2$), 6.73 (dd, *J* = 8.6 and 1.0 Hz, 2H, 9′-H), 6.97 (d, *J* = 8.1 Hz, 2H, 2,7-H), 7.47–7.49 (m, 2H, 7′-H), 7.52–7.54 (m, 2H, 3,6-H), 7.56–7.59 (m, 2H, 8′-H), 7.67 (d, *J* = 7.8 Hz, 2H, 4,5-H), 8.12 (dd, *J* = 8.1 and 1.4 Hz, 2H, 6′-H); for ^{1}H-NMR analysis in the presence of the chiral shift reagent Eu(TFC)$_3$[20] see above. – $C_{36}H_{24}N_8O_4$: Calcd. 632.1921 (M^+), found 632.1960 (MS); Calcd. 633.1999 (MH^+), found 633.1953 (MS).

X-Ray Analysis of anti-1,8-Bis(3′-methylisoalloxazin-10′-yl)-9,10-dihydroanthracene (**26**): **26** crystallized from dichloromethane with inclusion of dichloromethane in a 1:2 ratio: orange rhombs (0.3 × 0.2 × 0.1 mm); $C_{36}H_{24}N_8O_4 \cdot 2\ CH_2Cl_2$; molecular mass 632.6 (+169.9 for 2 CH_2Cl_2); triclinic space group $P\bar{1}$ (No. 2, Intern. Tables); cell parameters: *a* = 1038.3(1), *b* = 1134.9(3), *c* = 1672.8(5) pm; α = 81.17(3)°, β = 76.26(2)°, γ = 71.71(2)°; *Z* = 2, D_x = 1.471 g · cm^{-3}; Enraf-Nonius CAD4 (Mo-K_α radiation, graphite monochromator, 2 Θ/Θ-scan technique); max. sin Θ/λ = 6.0 nm^{-1}; 6352 symmetry-independent reflections out of which 4284 with $I \geq 3.0\ \sigma(I)$ were classified as observed. The structure was solved by direct method (SIR); the refinement including all non-hydrogen atoms with anisotropic and the hydrogen atoms with isotropic temperature factors resulted in *R* = 0.061. For atomic coordinates and equivalent isotropic thermal parameters for the non-hydrogen atoms see ref.[28]. The structure of **26** is discussed above.

10-(5′,6′,7′,8′-Tetrahydro-1′-anthryl)-3-methylisoalloxazine (**27**): 12.6 mg (6.9%), orange needles of m. p. 295°C (dec. above 240°C). – MS: *m/z* (%) = 408 (100, M^+), 407 (54), 323 (20), 322 (25), 229 (15), 165 (15). – ^{1}H NMR (500 MHz, CD_2Cl_2; assignment by COSY): δ = 1.72–1.83 (m, 4H, 6′,7′-CH$_2$), 2.64–2.78 (m, 2H, 8′-CH$_2$), 2.95–2.99 (m, 2H, 5′-CH$_2$), 3.38 (s, 3H, 3-CH$_3$), 6.74–6.76 (m, 1H, 9-H), 6.81 (s, 1H, 9′-H), 7.33 (d, *J* = 7.3 Hz, 1H, 2′-H), 7.55–7.58 (m, 2H, 7,8-H), 7.60–7.62 (m, 1H, 3′-H), 7.76 (s, 1H, 10′-H), 8.03 (d, *J* = 8.4 Hz, 1H, 4′-H), 8.31–8.33 (m, 1H, 6-H). – $C_{25}H_{20}N_4O_2$: Calcd. 408.1586 (M^+), found 408.1651 (MS).

10-(1′-Naphthyl)isoalloxazine: 500 mg (2.13 mmol) of 1-(2′-aminophenylamino)naphthalene[24] was treated with 750 mg (4.68 mmol) of alloxan hydrate and 1.25 g (20.23 mmol) of boric acid in 75 ml of acetic acid (2 h, room temp., under argon). Then the reaction mixture was poured into 300 ml of water and extracted five times with 70 ml of trichloromethane. The combined extracts were dried with anhydrous magnesium sulfate, and the solvent was evaporated. The residue was crystallized from methanol to afford 650 mg (yield 90%) of an orange powder, dec. > 300°C; $R_f \approx 0.36$ (silica gel; trichloromethane/methanol, 10:1). – MS: *m/z* (%) = 340 (100, M^+), 339 (64), 269 (93), 268 (73), 135 (16), 127 (23). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 6.51 (dd, *J* = 8.3 and 2.0 Hz, 1H, 9-H), 7.47–7.49 (m, 1H, 3′-H), 7.58–7.79 (m, 5H, 7,8,2′,7′,8′-H), 7.80–7.82 (m, 1H, 6′-H), 8.17 (d, *J* = 8.3 Hz, 1H, 6-H), 8.23–8.27 (m, 2H, 4′,5′-H), 11.40 (br. s, 1H, NH). – $C_{20}H_{12}N_4O_2$ (340.33): Calcd. C 70.58, H 3.55, N 16.46; found C 70.39, H 3.46, N 16.20.

3-Methyl-10-(1′-naphthyl)isoalloxazine (**28**): A suspension of 390 mg (1.15 mmol) of 10-(1′-naphthyl)isoalloxazine, 1.58 g (11.5 mmol) of potassium carbonate and 0.71 ml (11.5 mmol) of iodomethane in 50 ml of dry DMF was stirred at room temp. for 20 h under argon. After addition of 400 ml of water the mixture was extracted four times with 70 ml each of dichloromethane, and the combined extracts were dried with anhydrous magnesium sulfate. Evaporation of the solvent and crystallization of the residue from acetonitrile afforded 310 mg (yield 76%) of yellow needles, dec. > 300°C. **28** had been prepared by a different method (m. p. 300–304°C, no further characteristic data were reported)[25]. – MS: *m/z* (%) = 354 (100, M^+), 353 (44), 269 (57), 268 (24), 127 (23). – ^{1}H NMR (500 MHz, [D$_6$]DMSO; assignment by COSY): δ = 3.26 (s, 3H, 3-CH$_3$), 6.36 (d, *J* = 8.8 Hz, 1H, 9-H), 7.46–7.48 (m, 1H, 3′-H), 7.55 (d, *J* = 8.4 Hz, 1H, 2′-H), 7.61–7.66 (m, 4H, 7,8,7′,8′-H), 7.80–7.82 (m, 1H, 6′-H), 8.18 (d, *J* = 8.3 Hz, 1H, 6-H), 8.26–8.29 (m, 2H, 4′,5′-H). – $C_{21}H_{14}N_4O_2$ (354.35): Calcd. C 71.18, H 3.98, N 15.81; found C 71.41, H 4.04, N 15.87.

1-(2′-Nitrophenylamino)anthracene: To a suspension of 8.5 g (129 mmol) of potassium hydroxide (85% KOH) in a solution of 5.0 g (25.9 mmol) of 1-aminoanthracene in 100 ml of dry DMSO 3.42 ml (32.3 mmol) of 2-fluoronitrobenzene was added dropwise under argon with cooling and vigorous stirring at 20–25°C. After 4 h at room temp. the reaction mixture was poured in 500 ml of water and acidified with 2 N hydrochloric acid. Fivefold extraction with 100 ml of dichloromethane, drying of the combined extracts with anhydrous magnesium sulfate and evaporation of the solvent yielded a residue which was dissolved in toluene; the solution was filtered through silica gel (2-cm layer). The product obtained was crystallized from toluene to yield 4.24 g (52%) of 1-(2′-nitrophenylamino)anthracene as purple needles, m. p. 214–215°C. – MS: *m/z* (%) = 314 (100, M^+), 280 (37), 269 (32), 268 (88), 267 (71), 266 (31). – ^{1}H NMR (500 MHz, [D$_6$]DMSO; assignment by COSY and NOE): δ = 6.70 (d, *J* = 8.6 Hz, 1H, 6′-H), 6.83–6.85 (m, 1H, 4′-H), 7.36–7.39 (m, 1H, 5′-H), 7.49–7.61 (m, 4H, 2,3,7,8-H), 8.08–8.13 (m, 3H, 4,6,9-H), 8.21 (dd, *J* = 8.6 and 1.2 Hz, 1H, 3′-H), 8.61 (s, 1H, 9-H), 8.71 (s, 1H, 10-H), 9.82 (br. s, 1H, NH). – $C_{20}H_{14}N_2O_2$ (314.33): Calcd. C 76.42, H 4.49, N 8.91; found C 76.70, H 4.49, N 8.85.

10-(1′-Anthryl)isoalloxazine: To a solution of 500 mg (1.59 mmol) of 1-(2′-nitrophenylamino)anthracene in 70 ml of acetic acid 2.50 g (38.2 mmol) of zinc dust was added at 60–70°C with vigorous stirring in small portions under argon. After 20 min the solution was filtered into a suspension of 400 mg (2.5 mmol) of alloxan hydrate and 1.5 g (24.3 mmol) of boric acid in 20 ml of acetic acid. After stirring for 2 h at room temp. and the addition of 500 ml of water, the solution was extracted five times with 70 ml each of dichloromethane. The combined extracts were dried with anhydrous magnesium sulfate, the solvent was evaporated, and the product was treated with 50 ml of boiling methanol to afford 233 mg (38%) of a brick-red crystalline powder, m. p. 245°C (dec.). – MS: *m/z* (%) = 390 (100, M^+), 389 (12), 320 (16), 319 (61), 318 (37), 160 (15). – ^{1}H NMR (500 MHz, [D$_6$]DMSO; assignment by COSY and NOE): δ = 6.60–6.62 (m, 1H, 9-H), 7.47–7.50 (m, 1H, 7′-H), 7.55–7.57 (m, 1H, 6′-H), 7.60–7.62 (m, 2H, 7,8-H), 7.66 (d, *J* = 6.9 Hz, 1H, 2′-H), 7.77–7.79 (m, 1H, 3′-H), 7.86 (d, *J* = 8.5 Hz, 1H, 8′-H), 8.17 (d, *J* = 8.5 Hz, 1H, 5′-H), 8.27–8.29 (m, 2H, 6,9′-H), 8.42 (d, *J* = 8.6, 1H, 4′-H), 8.85 (s, 1H, 10′-H), 11.45 (br. s, 1H, NH). – $C_{24}H_{14}N_4O_2$ (390.38): Calcd. C 73.84, H 3.61, N 14.35; found C 73.66, H 3.86, N 14.28.

10-(1′-Anthryl)-3-methylisoalloxazine (**29**): A suspension of 170 mg (0.44 mmol) of 10-(1′-anthryl)isoalloxazine, 1.20 g (8.71 mmol) of potassium carbonate and 0.54 ml (8.71 mmol) of iodomethane in 70 ml of dry acetone was stirred for 18 h at room temp. under argon. After evaporation of the solvent the residue was dissolved in dichloromethane and the solution filtered through Celite. Evaporation of the solvent from the filtrate and crystallization of the residue from acetic acid yielded 120 mg (68%) of **29** as a brick-red crystalline powder, m. p. 250°C (dec.). – MS: *m/z* (%) = 404 (100, M^+), 403 (32), 320 (22), 319 (88), 318 (58). – ^{1}H NMR (500 MHz, [D$_6$]DMSO; assignment by COSY): δ = 3.27 (s, 3H, 3-CH$_3$), 6.64–6.66 (m, 1H, 9-H), 7.48–7.51 (m, 1H, 7′-H), 7.55–7.57 (m, 1H, 6′-H), 7.61–7.64 (m, 2H, 7,8-H), 7.66 (d, *J* = 7.0 Hz, 1H, 2′-H), 7.78–7.80 (m, 1H, 3′-H), 7.84 (d, *J* = 8.5 Hz, 1H, 8′-H), 8.17 (d, *J* = 8.5 Hz, 1H, 5′-H), 8.25 (s, 1H, 9′-H), 8.32–8.34 (m, 1H, 6-H), 8.43 (d, *J* = 8.6 Hz, 1H, 4′-H), 8.86 (s, 1H, 10′-H). – $C_{25}H_{16}N_4O_2$ (404.43): Calcd. C 74.24, H 3.99, N 13.86; found C 73.98, H 3.81, N 13.82.

[1] Part 2: M. F. Zipplies, H. A. Staab, *Tetrahedron Lett.* **1984**, *25*, 1035–1038. – Part 1: M. F. Zipplies, C. Krieger, H. A. Staab, *ibid.* **1983**, *24*, 1925–1928.

[2] T. Iyanagi, N. Makino, H. S. Mason, *Biochemistry* **1974**, *13*, 1701–1710.

[3] R. Kuhn, R. Ströbele, *Ber. Dtsch. Chem. Ges.* **1937**, *70*, 753–760.

[4] Cf. H. Beinert, *J. Am. Chem. Soc.* **1956**, *78*, 5323–5328; V. Massey, G. Palmer, *J. Biol. Chem.* **1962**, *237*, 2347–2358.

[5] P. Hemmerich, V. Massey, H. Michel, C. Schug, *Scope and Limitations of Single Electron Transfer in Biology*, in *Structure and Bonding* **48**, Springer Verlag, Berlin–Heidelberg–New York **1982**, pp. 93–123.

[6] a) N. J. Leonard, R. F. Lambert, *J. Org. Chem.* **1969**, *34*, 3240–3248; b) Y. Yano, E. Ohya, *Chem. Lett.* **1983**, 1281–1284; *J. Chem. Soc. Perkin Trans 2*, **1984**, 1227–1232; c) P. J. H. Bastiaens, A. J. W. G. Visser, in *Flavins and Flavoproteins* **1990**, (B. Custi, S. Ronchi, G. Zanetti, eds.), Walter de Gruyter & Co., Berlin–New York **1991**, pp. 73–78.

[7] H. A. Staab, M. F. Zipplies, T. Müller, M. Storch, C. Krieger, *Chem. Ber.* **1994**, *127*, 1667–1680, following paper.

[8] H. A. Staab, J. Kanellakopoulos, C. Krieger, P. Kirsch, paper to be published.

[9] H. A. Staab, W. Rebafka, *Chem. Ber.* **1977**, *110*, 3333–3350; H. A. Staab, C. P. Herz, C. Krieger, M. Rentea, *ibid.* **1983**, *116*, 3813–3830; further references in H. A. Staab, *New Aspects of Organic Charge-Transfer Compounds*, in *New Aspects of Organic Chemistry I* (Z. Yoshida, T. Shiba, Y. Ohshiro, eds.), VCH Weinheim–New York–Cambridge–Basel **1989**, Chapter 10.

[10] H. A. Staab, R. G. H. Kirrstetter, *Liebigs Ann. Chem.* **1979**, 886–898; H. A. Staab, N. Riegler, F. Diederich, C. Krieger, D. Schweitzer, *Chem. Ber.* **1984**, *117*, 246–259.

[11] H. A. Staab, J. Weiser, E. Baumann, *Chem. Ber.* **1992**, *125*, 2275–2283; H. A. Staab, J. Weiser, M. Futscher, G. Voit, A. Rückemann, C. Anders, *ibid.* **1992**, *125*, 2285–2301; H. A. Staab, G. Voit, J. Weiser, M. Futscher, *ibid.* **1992**, *125*, 2303–2310; C. Krieger, M. Dernbach, G. Voit, T. Carell, H. A. Staab, *ibid.* **1993**, *126*, 811–821; further references therein.

[12] See, for example, P. Wahl, C. Krieger, D. Schweitzer, H. A. Staab, *Chem. Ber.* **1984**, *117*, 260–276.

[13] Cf. F. Vögtle, P. Neumann, *Tetrahedron* **1970**, *26*, 5847–5873.

[14] **12** was independently prepared by our group (ref.[1]) and by Y. Yano and E. Ohya[6b]; since in these papers no further synthetic and analytical data were reported such information is presented here.

[15] F. Gückel, D. Schweitzer, K. Becker, R. H. Schirmer, M. F. Zipplies, H. A. Staab, in *Flavins and Flavoproteins* **1987** (D. E. Edmondson, D. B. McCormick, eds.), Walter de Gruyter & Co, Berlin–New York **1987**, pp. 59–67.

[16] W. Rupe, D. Wasserzug, *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 3527–3531; P. Klinke, H. Gibian, *Chem. Ber.* **1961**, *94*, 26–38.

[17] F. Yoneda, Y. Sakuma, M. Ichiba, K. Shinomura, *J. Am. Chem. Soc.* **1976**, *98*, 830–835.

[18] R. Kuhn, F. Weygand, *Ber. Dtsch. Chem. Ges.* **1935**, *68*, 1282–1288.

[19] P. Kirsch, Ph. D. Thesis, University of Heidelberg, **1993**.

[20] Eu(TFC)$_3$ = Tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-D-camphorato]europium.

[21] For a recent alternative preparation of 1,8-diaminoanthracene cf. J. L. Sessler, T. D. Mody, D. A. Ford, V. Lynch, *Angew. Chem.* **1992**, *104*, 461–464; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 452–455.

[22] N. R. Rao, K. H. Shah, K. Venkataraman, *Proc. Indian Acad. Sci., Sect. A.*, **1951**, *34*, 355–364.

[23] P. Hemmerich, *Helv. Chim. Acta* **1964**, *47*, 464–475; W. B. Cowden, P. K. Halladay, R. B. Cunningham, N. H. Hunt, I. A. Clark, *J. Med. Chem.* **1991**, *34*, 1818–1822.

[24] W. C. J. Ross, *J. Chem. Soc.* **1948**, 219–224.

[25] S. Shinkai, H. Nakao, I. Kuwahara, M. Miyamoto, T. Yamaguchi, O. Manabe, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 313–319.

[26] Cf. H. A. Staab, B. Starker, C. Krieger, *Chem. Ber.* **1983**, *116*, 3831-3845; further references therein.

[27] P. Hemmerich, *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 451–527.

[28] Further details of the crystal structure investigations are available on request from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information mbH, D-76344 Eggenstein-Leopoldshafen, on quoting the depositary number CSD-58194, the names of the authors, and the journal citation.

[29] H. Kiesele, *Anal. Chem.* **1980**, *52*, 2230–2232.

[76/94]