π···π Interactions of Flavins, 4[1]

Pyridinio-isoalloxazinophanes as Model Systems for Active-Site Complexes in Flavoenzymes: Syntheses, X-Ray Structure Analyses and Spectroscopic Properties

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As model systems for active site complexes in flavoenzymes, flavin and nicotinamide analogues were linked together in cyclophane skeletons of specific sterical structures. Elaborating this concept, we prepared [4]metacyclo[3](10,6)isoalloxazinophane (**3**), [4](3,1)pyridino[3](10,6)isoalloxazinophane (**4**), as well as the 16-methoxycarbonyl and the 16-carboxylato derivatives (**2** and **34**, resp.), of **4** by multistep syntheses. For the isoalloxazinophanes **2**, **3**, **4**, and **34** X-ray structure analyses were performed and are discussed with regard to intramolecular interactions. Preliminary UV/Vis-spectroscopic results related to π···π interactions in these isoalloxazinophanes are reported.

The important role flavins play "at the crossroad of biological redox chemistry"[2] is directly related to the fact that flavins represent a versatile system of various redox states and are capable of transferring one-electron as well as two-electron equivalents. As a consequence of these specific properties, flavin coenzymes – flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD) – often act in enzymatic reactions as mediators between otherwise incompatible redox systems. For example, FAD in glutathione reductase catalyzes the two-electron reduction of oxidized glutathione by dihydronicotinamide-adenine dinucleotide phosphate (NADPH). The X-ray structure analysis of glutathione reductase[3] and the subsequent investigation of mechanistic details of this enzyme as well as of the geometry of its active site[4] stimulated further interest with regard to interactions between flavin and nicotinamide units.

Open-chain trimethylene-linked flavin-nicotinamide systems were synthesized already in the mid-70s, and charge-transfer interactions between flavin and nicotinamide in their respective reduced and oxidized states were studied[5]. The high flexibility of these open-chain models, however, did not allow the observed effects to be ascribed precisely to specific mutual orientations of the interacting units.

In order to limit the conformational diversity we tried to obtain less flexible systems by a twofold bridging from the 5- and 6-positions of nicotinamide to the 6- and 9-positions of the isoalloxazine unit by tetramethylene chains[6]. Whereas these attempts, due to synthetic difficulties, were not successful yet, we report in this paper on syntheses and properties of nicotinamide-flavin models in which the interacting components are kept in a rigid face-to-face orientation by a suitable cyclophane skeleton. The "cyclophane concept" had been successfully used, for example, for studying the redox equivalent exchange between two nicotinic ester units of different redox states[7], for investigating flavin-flavin interactions[1], and more generally for studying charge-transfer[8] and excimer[9] interactions as well as, rather recently, photoinduced electron-transfer reactions in porphyrin-quinone cyclophanes[10].

In the context of the problems discussed above, the specific cyclophanes wanted were the nicotinamide-isoalloxazinophane **1** and the corresponding nicotinic ester **2** which with regard to transannular interactions is a close analogue of **1**. Fixed in the rigid cyclophane skeleton the pyridinio-isoalloxazine arrangement in **1** and **2** would correspond to the alignment of the reactants as derived for the active site of glutathione reductase. As preparatory steps for designing and testing the synthetic approach to these flavinocyclophanes and for exploring whether at all a rigid parallel arrangement can be achieved by this type of a cyclophane skeleton, [4]metacyclo[3](10,6)isoalloxazinophane (**3**) and the corresponding [4](3,1)pyridinio[3](10,6)-isoalloxazinophane system **4** were first to be synthesized, and their structures were to be determined.

Syntheses and Characterizations of Isoalloxazine Cyclophanes

[4]Metacyclo[3](10,6)isoalloxazinophane (**3**): In contrast to more conventional routes to cyclophanes, in the case of flavinocyclophanes due to the sensitivity of the isoalloxazine system it was necessary to build up the macrocyclic system first and to complete the synthesis of the isoalloxazine unit at the end of the synthetic sequence. Thus, a key intermediate *en route* to **3** was 20-amino-1-aza-[4.4]metacyclophane (**5**) containing the *ortho*-phenylenediamine constituent which by condensation with alloxan[11] should yield **3**. The strategy for synthesizing **5** was expected

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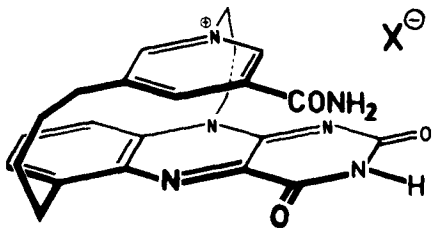

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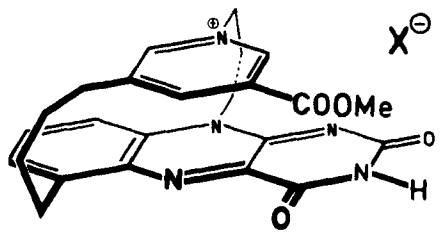

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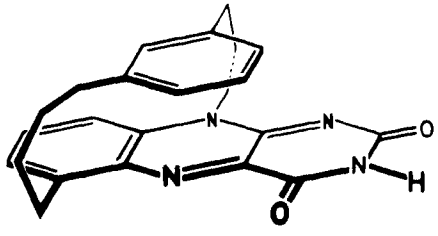

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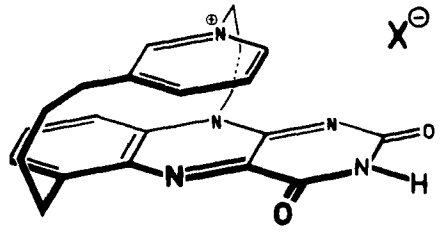

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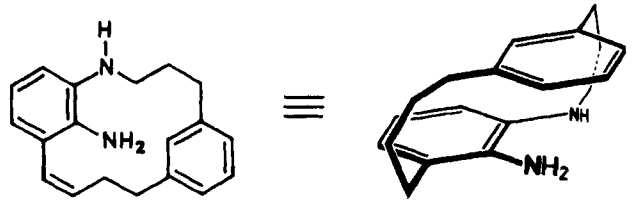
to consider that, in principle, it should be applicable as well to the syntheses of the other flavin cyclophanes mentioned.

The preparation of **5**[12] started from 3-acetylaminobenzoic acid, the nitration of which and the separation of the isomers formed were accomplished by an improved procedure in analogy to ref.[13] (see Experimental). 3-Acetylamino-2-nitrobenzoic acid either *via* the corresponding benzoyl chloride or by intermediate formation of the mixed anhydride with ethyl chloroformate[14], and subsequent reduction by sodium borohydride yielded 3-acetylamino-2-nitrobenzyl alcohol (**19**) (overall yield 62 and 75%, respectively). By oxidation with pyridinium chlorochromate 3-acetylamino-2-nitrobenzaldehyde (**6**) (yield 56%) was obtained as the first component for the envisaged Wittig reaction. As the second component, 1-(3-bromopropyl)-3-(3-triphenylphosphoniopropyl)benzene bromide (**7**) was obtained by treatment of 1,3-bis(3-bromopropyl)benzene[15] with triphenylphosphane (yield 63%).



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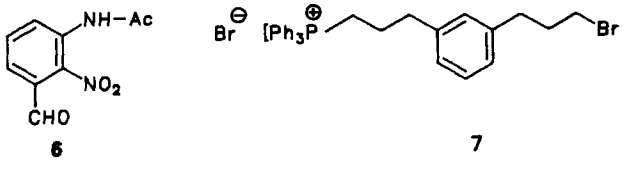
The Wittig reaction of **6** with **7** (potassium *tert*-butylate, THF, −78°C; for details see Experimental) resulted in the formation of a 7:3 mixture of the *Z* and *E* isomers of 1-(3-acetylamino-2-nitrophenyl)-4-[3-(3-bromopropyl)phenyl]-butene-1 (**8** and **9**, resp.; overall yield 68%). The isomers were separated by column chromatography; the assignment was based on ^{1}H NMR. Cyclization of the *Z* isomer by dropwise addition of a diluted solution of **8** in toluene to a refluxing phase-transfer system of toluene/aqueous sodium hydroxide (2.5%)/tetra-*n*-butylammonium bromide yielded (*Z*)-1-acetyl-20-nitro-1-aza[4.4]metacyclophan-13-ene (**10**) (yield ≈ 85%). From the *E* isomer **9** under similar cyclization conditions a macrocyclic product with twice the molecular mass was obtained for which all analytical and spectroscopic data are in accordance with the [4.4.4.4]metacyclophane structure **11** (for details see Experimental). Basic hydrolysis of compound **10** (potassium hydroxide, ethanol/water; 70 min at 85–90°C, under argon) yielded the deacetylated **12** (coral-red needles, yield 79%).



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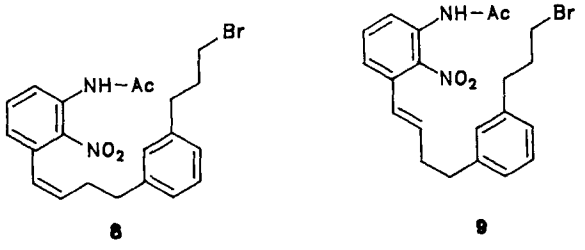

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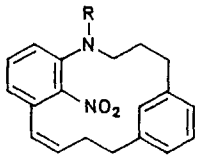
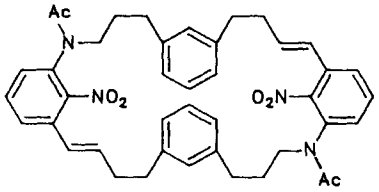
Catalytic hydrogenation of nitro group and double bond resulted in the formation of **5** which due to its instability was not isolated but was immediately treated with alloxan hydrate in boric acid/acetic acid to afford **3** in 71% yield as deep yellow crystals. Elemental analysis and mass spectrum support the structure postulated. ^{1}H-NMR data, assigned for the majority of protons by decoupling and NOE experiments (see Experimental), are in agreement with the spatial structure as shown in formula **3**. Especially the remarkable

upfield shift to δ = 5.67 for 19-H, the aromatic proton between the methylene bridges, is in accordance with a nearly parallel arrangement of this ring above the central section of the isoalloxazine unit, thus positioning this proton into the shielding region of the lateral aromatic ring of the isoalloxazine system. The X-ray structure analysis obtained for **3** (see below) confirms the sandwich-type structure and reveals by short transannular distances that there is considerable overlap between the two π systems.



10: R = Ac
12: R = H



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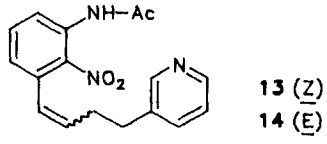
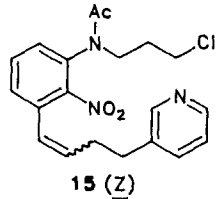
For **3**, as for the related flavinocyclophanes, a modified atom numbering is used differing from the conventional cyclophane nomenclature rules in so far as for the isoalloxazine units the usual flavin numbering is retained (see Figure 2 in which the molecular structure of **3** is presented).

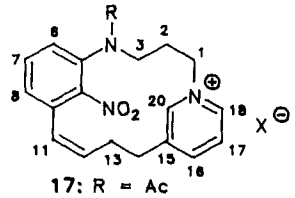
[4](3,1)Pyridinio[3](10,6)isoalloxazinophane Chloride and Perchlorate (**4**, X=Cl and X=ClO_4): For building up the pyridinio analogue **4** of [4]metacyclo[3](10,6)isoalloxazinophane (**3**) the synthesis followed in principle the concept of the preparation of **3**. The Wittig reaction leading to **13**/**14** required in addition to the aldehyde **6** as the second component 3-(3-triphenylphosphoniopropyl)pyridine bromide which was prepared by reaction of 3-(3-hydroxypropyl)pyridine with triphenylphosphane in the presence of hydrobromic acid (yield 84%). From this phosphonium salt the ylide was obtained which by treatment with **6** (for details see Experimental) yielded a mixture of *Z*/*E* isomers **13** and **14** the chromatography of which led only to partial separation (assignment by ^{1}H-NMR spectrometry).



13 (Z)
14 (E)



15 (Z)
16 (E)

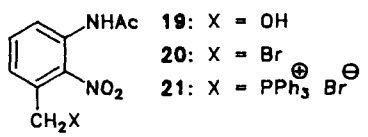


17: R = Ac
18: R = H

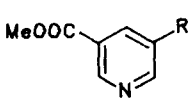
The *Z*/*E* isomer mixture (**13**/**14**) containing about 90% of the wanted isomer **13** by reaction with 1-bromo-3-chloropropane (potassium hydroxide, dry DMSO) yielded after chromatography (silica gel, ethyl acetate) the *N*-(3-chloropropyl) derivatives **15** and **16** (ratio ca. 9:1, total yield 85%). The cyclization of **15** to **17** was achieved by intermediate nucleophilic replacement of the chloro by the iodo substituent and subsequent intramolecular *N*-alkylation of the pyridine unit to **17**(X=I) (potassium iodide, DMF/acetone, high dilution; yield 58%). Analytical and spectroscopic data are in full accordance with the structure of **17**(X=I) as well as with the corresponding chloride **17**(X=Cl) which was obtained from the iodide by anion exchange (for details see Experimental). Splitting off the *N*-acetyl group of **17**(X=Cl) was achieved by acidic hydrolysis (concd. hydrochloric acid, heating at reflux for 90 min under argon); the deacylation product **18**(X=Cl) was obtained in 88% yield as brick-red crystals.

Hydrogenation of the nitro group and the double bond of **18** should lead to the pyridinio analogue of **5**, containing the *ortho*-phenylenediamine unit required for the alloxan condensation to the pyridinio-isoalloxazinophane **4**. Due to its oxidation sensitivity it did not seem advisable to isolate the intermediate *ortho*-phenylenediamine. On the other hand, the immediate succession of the two reaction steps turned out to be difficult because of an incompatibility of the reaction conditions. In an extended series of experiments[16] the following procedure was found to lead to optimal results: The hydrogenation of **18**(X=Cl) was performed on palladium oxide catalyst in aqueous hydrochloric acid ($c \approx 1$ mol·l^{-1})/methanol (theoretical hydrogen uptake at 20°C/1 atm. after ca. 50 min); the solution was then directly introduced into a suspension of alloxan hydrate in concd. hydrochloric acid, and the reaction mixture was heated to 70–95°C for 2 h. Working up by extraction of alloxan with 1-butanol, gel-permeation chromatography (Sephadex LH 20, methanol; DC-control, $R_f \approx 0.25$) and crystallization from ethanol/diethyl ether yielded yellow needles (dec. > 251°C; 27–43%). The elemental analysis of the hygroscopic product was in accordance with the presence of a monohydrate of **4**(X=Cl). Filtration through perchlorate-loaded ion-exchange column yielded **4**(X=ClO_4) with correct elemental analysis. Mass spectrometric fragmentations agree with the pyridinio-isoalloxazinophane structure **4** as does the ^{1}H-NMR spectrum (360 MHz, [D_6]DMSO). On the basis of decoupling experiments and the comparison of chemical shifts with those of the precursor and of **3**, all ^{1}H-NMR signals of the seven "aromatic" protons (pyridinium system and lateral benzene ring of isoalloxazine) of **4** were unambiguously assigned. Of special interest with regard to the sterical structure of **4** is the shielding effect observed for all protons of the pyridinium system as compared to the corresponding protons of 1,3-dimethylpyridinium salts containing the same substitution pattern as **4**. This effect is strongest for 19-H ($\Delta\delta \approx 1.0$) due to the fact that this proton is especially exposed to the ring-current effect of the lateral aromatic ring of the isoalloxazine unit. Thus, as discussed for **3**, a cyclophane conformation with an approximately parallel "sandwich" orientation of the pyridinio and the isoalloxazine units is strongly supported by ^{1}H-NMR spectrometry. As will be shown below, this conformation has been confirmed by an X-ray structure analysis of **4**(X=ClO_4).

[4](3,1)Pyridinio[3](10,6)isoalloxazinophanes Containing Nicotinic Acid Derivatives as Pyridinio Units (**2** and **34**): Synthesis and structure determination of **4** proved that the specific cyclophane skeleton wanted for **1** and **2** is obtainable. The synthesis of these specific nicotinic derivatives, however, required a somewhat modified strategy. For coupling the precursor components of the flavin and nicotinic subunits the decisive Wittig reaction had to be carried out with reversed functionalities, the coupling components being the triphenylphosphonium bromide **21** and 3-(5-methoxycarbonyl-3-pyridyl)propanal (**24**). By reaction of 3-acetylamino-2-nitrobenzyl alcohol (**19**, see above) with triphenylphosphane/tetrabromomethane[17] (yield 65%) the corresponding benzyl bromide **20** was obtained which with excess triphenylphosphane yielded the phosphonium bromide **21** (yield 98%).



19: X = OH
20: X = Br
21: X = $PPh_3^{\oplus}$ $Br^{\ominus}$

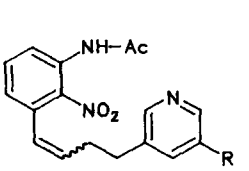


22: R = CH=CH−CH(OMe)$_2$
23: R = $CH_2-CH_2-CH(OMe)_2$
24: R = CH_2-CH_2-CHO

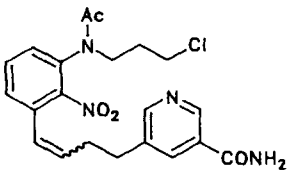
For the synthesis of the aldehyde component **24**, methyl 3-bromopyridine-5-carboxylate[18] was treated with acrolein dimethyl acetal to yield **22** in a Heck reaction[19] [palladium(II) acetate, triethylamine, tri-*o*-tolylphosphane, acetonitrile, 16h reflux under argon, yield ca. 18%]. Compound **22** was hydrogenated to **23** which by acidic cleavage formed **24** (for details see Experimental).

The Wittig reaction of **21** with **24** was performed according to a variant reported in ref.[20] (potassium fluoride, dibenzo-[18]crown-6, acetonitrile, 10h at 80°C) yielding **25** as *Z*/*E* mixture (ca. 3:2; overall yield 87%); by chromatography and fractional crystallization the *Z* and *E* isomers were further enriched. For the following alkylation and cyclization steps the methoxycarbonyl compound **25** had to be converted into the less sensitive carbamoyl derivative **26** (ammonia, methanol/water; yield 80%, *Z*/*E* ratio ca. 6:1). The isomer mixture of **26** was treated with 1-bromo-3-chloropropane to give **27** (yield 89%, *Z*/*E* ratio 4:1, after chromatography).

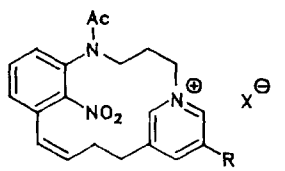
The cyclization of **27** (*Z*/*E* = 4:1) was achieved by dropwise addition at high dilution into a refluxing solution of potassium iodide in DMF/acetone. By extraction, chromatography and recrystallization (see Experimental) the cyclization product **28** was obtained. By anion exchange the corresponding chloride **29** and bromide **30** were prepared. For the monohydrate of the latter an X-ray structure analysis (see below) was performed. The molecular structure shows for **30** the presence of an *anti* conformation of the aza[4.4]-metacyclophane skeleton with the ring systems and substituents already in positions especially suited for the isoalloxazinophane synthesis to follow later (see Figure 1).



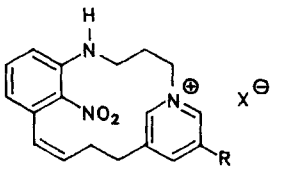
25 (Z,E): R = COOMe
26 (Z,E): R = $CONH_2$



27 (Z,E)



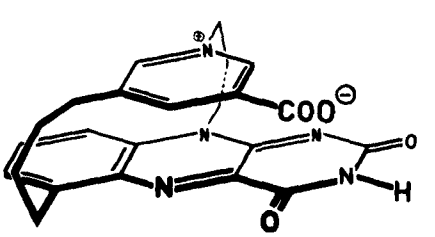
28: R = $CONH_2$, X = I
29: R = $CONH_2$, X = Cl
30: R = $CONH_2$, X = Br



31: R = $COO^{\ominus}$ (no X)
32: R = COOMe, X = Br
33: R = $CONH_2$, X = Br

For the preparation of the pyridinio-isoalloxazinophanes by alloxan condensation the *N*-acetyl group on the macrocyclic ring was to be split off whereas the carboxamide group in the nicotinamide unit should be preserved. In fact, however, acidic hydrolysis of **29** resulted in the cleavage of the two amide bonds in **28** leading to the zwitterionic system **31** (yield 71%) which later proved to be of special interest as a precursor of the corresponding zwitterionic carboxylato-pyridinio-isoalloxazinophane **34** (see below). A stepwise conversion to **33** was achieved by treating **29** with acetyl chloride (5%) in methanol at reflux for 18h to yield, after anion exchange and recrystallization from water, the methoxycarbonyl compound **32**. The nicotinic amide unit was successfully reconstructed by treating **32** with a saturated solution of ammonia gas in methanol (orange crystals, yield 70%).

As the final steps to the pyridinio-isoalloxazinophanes the hydrogenation of the nitro group and the double bond and the subsequent condensation of the *ortho*-phenylenediamine part of the cyclophane with alloxan did not yet succeed with **32** and **33**. When we started from the zwitterionic **31**, however, catalytic hydrogenation immediately followed by condensation with alloxan hydrate in concd. hydrochloric acid resulted after extensive purification by chromatography and recrystallization from water/acetone in the isolation of the pure 16-carboxylato[4](5,1)pyridinio-[3](10,6)isoalloxazinophane (**34**). This nicotinate-flavinophane was characterized by high-resolution FAB mass spectrometry of the molecular ion, by complete assignment of ^{1}H-NMR data (using COSY and ROESY) and by an X-ray structure analysis (see below).



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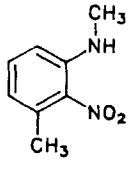
The synthesis of **34**, containing the complete carbon skeleton of **1** and **2**, now was expected to provide an alternative access to these flavinophanes with nicotinic ester and nicotinamide units. Indeed, when **34** was heated in a mixture of methanol (5%) and acetyl chloride (3h at reflux) 16-methoxycarbonyl[4](5,1)pyridinio[3](10,6)isoalloxazinophane chloride **2(X=Cl)** was obtained as fine yellow needles (yield 35%). The spectroscopic data are fully in accordance with the structure proposed. For the corresponding iodide **2(X=I)** obtained from the chloride **2** by anion exchange the detailed structure will be discussed below on the basis of an X-ray structure analysis.

So far all attempts to obtain the nicotinamide-isoalloxazinophane **1** itself by ammonolysis of **2** failed. Under the reaction conditions which led to the formation of **33** from **32**, the pyridinio-isoalloxazinophane **2** remained unchanged. Most probably it is the sterical shielding by the polar part of the closely neighboring isoalloxazine unit which in this case prevents the conversion of the nicotinic ester into the nicotinamide. Since, however, the electronic effects of the methoxycarbonyl and the carbamoyl substituents on the π system of the pyridine unit are rather similar, the π···π interaction in the flavinophane **1** can be expected to correspond closely to that of **2**.

6,10-Dimethylisoalloxazine (**36**): All isoalloxazinophanes reported in this study contain isoalloxazine units with alkyl substituents in the 6- and 10-positions. As reference compound for spectroscopic studies, therefore, 6,10-dimethylisoalloxazine (**36**) was prepared. For this purpose 3-methyl-2-nitroaniline[22] was acetylated to the corresponding acetanilide (yield 85%). *N*-Methylation was achieved with iodomethane, and the *N*-acetyl group was split off by basic hydrolysis to yield 3-(*N*-methylamino)-2-nitrotoluene (**35**) (orange-yellow needles, yield 90%). Catalytic hydrogenation immediately followed by reaction with alloxan hydrate yielded after chromatographic purification (see Experimental) **36** (yellow needles, yield 37%).



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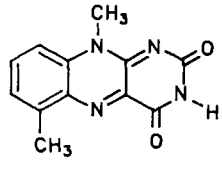

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X-Ray Structure Analyses of the 4-Aza[4]metacyclo[4]pyridiniophane Bromide 30 and of the Isoalloxazinophanes 2, 3, 4 and 34

Of the 4-aza[4]metacyclo[4]pyridiniophane bromide **30** and of the isoalloxazinophanes **3**, **4(X=ClO4)**, **2(X=I)** and **34** crystals suitable for X-ray structure analyses were obtained. The crystal and data collection parameters for these compounds are listed in Table 2. The data were collected by an Enraf-Nonius CAD 4 diffractometer (Mo-K_α radiation, graphite monochromator, 2Θ/ω scan). The structure solutions of **3**, **4(X=ClO4)**, and **34** were achieved by direct methods (MULTAN, SIR); the solution of the structures of **30** and **2(X=I)** made use of the heavy atom method (Patterson); an empirical absorption correction was applied to **2(X=I)** (Ψ-scan) and to **30**.

For the refinement all non-hydrogen atoms were included with anisotropic temperature factors and all hydrogen atoms with isotropic temperature factors. For further details including lists of atomic coordinates, estimated standard deviations and anisotropic temperature factors as well as of bond distances and bond angles see ref.[23].

(11Z)-4-Acetyl-17-carbamoyl-10-nitro-4-aza[4]metacyclo[4](5,1)pyridiniophan-11-ene Bromide (**30**): This substituted 4-aza[4]metacyclo[4]pyridiniophane is a typical representative of a precursor for the syntheses of isoalloxazinophanes by subsequent deacylation, hydrogenation and alloxan condensation. In this context, it is of interest that the [4.4]metacyclophane system of **30** is present in the *anti* conformation in which N(4) and N(10′) are in positions suitable for the alloxan condensation and already close to those they occupy later in the pyridinio-isoalloxazinophanes (see side view of **30** in Figure 1). In the unit cell two molecules of **30** are dimerized across a center of symmetry by two N−H···O hydrogen bonds of the carboxamide groups on C(17); the crystal water molecule is linked by an O−H···O hydrogen bond to the carbonyl oxygen of the acetyl group on N(4).

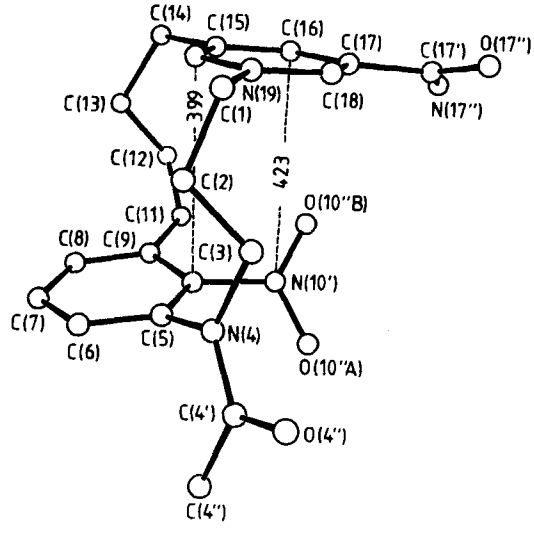

Figure 1. Molecular structure of **30** in a side view with relevant transannular distances

[4]Metacyclo[3](10,6)isoalloxazinophane (**3**): Figure 2 (A) shows the molecular structure of **3** in a top view onto the isoalloxazine plane. Bond lenghts and valence angles demonstrate that the incorporation of the isoalloxazine unit into the [3.4]cyclophane system does not significantly interfere with the normal isoalloxazine structure (such an interference would have limited the value of this series of isoalloxazinophanes as model systems for π···π interactions). As was already concluded from ^{1}H-NMR spectra (see above) the benzene ring is positioned vis-a-vis the central ring of the isoalloxazine system, although not in a fully ecliptic position. Both the flavin system and the benzene ring are nearly planar, the angle between the two planes being ca.

15°. With regard to π···π interactions the transannular atom-to-atom distances are of interest. As is shown in the side view [Figure 2 (B)] the shortest distance with 309 pm is found for C(14)···N(10). The packing of **3** in the crystal is dominated by a lateral dimerization of the molecules which occurs by two strong N(3)-H···O(2′) hydrogen bonds across a center of symmetry [*d*(N···O): 284.1(2) pm, ∢ 172(2)°]. There is a vertical (benzene-isoalloxazine) stacking in the crystal for which, however, no especially short intermolecular distances are observed.

[4](3,1)Pyridinio[3](10,6)isoalloxazinophane Perchlorate (**4, X = ClO$_4$**): Crystals of **4**-perchlorate containing in a 1:3 ratio crystal water were obtained by crystallization from water. To prevent the collapse of the crystal by loss of water, the crystallographic data collection had to be done in a sealed capillary containing the crystal in the aqueous mother liquor. In addition to two **4** ions and two perchlorate ions, the unit cell contains six water molecules obviously stabilizing the crystal by a network of hydrogen bonds involving N(3)−H of the isoalloxazines as well as perchlorate oxygens between the water molecules, the latter forming a partially disordered chain parallel to the crystallographic *b* axis. The two cyclophane units in the elementary cell are oriented to each other centrosymmetrically with the isoalloxazine parts parallel to each other and overlapping with their polar pyrimidinedione substructures at a distance of only about 315 pm.

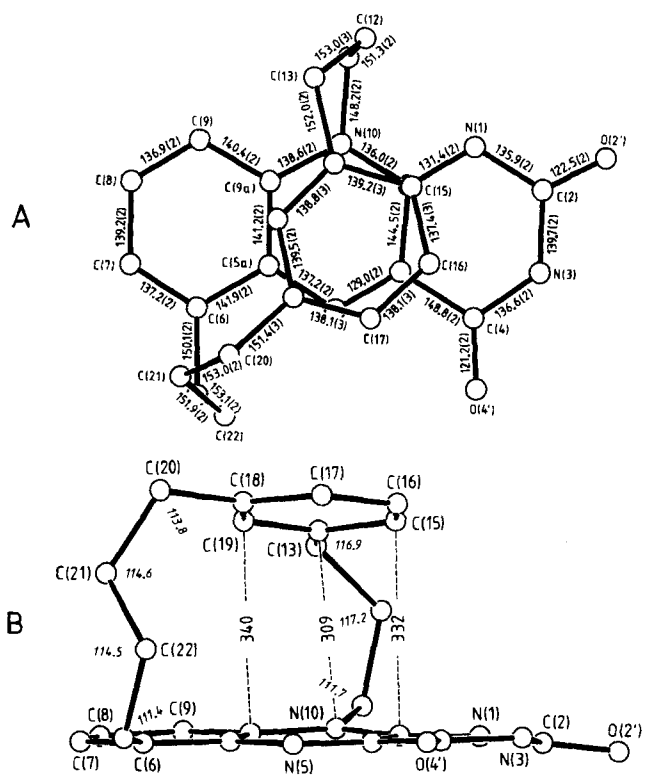

Figure 2. Molecular structure of **3** in a top view onto the isoalloxazine plane (A) and in a side view (B)

In **4** the isoalloxazine ring system and the pyridinium unit are nearly planar and almost parallel to each other with an interplanar angle of 9.0(4)° and an average distance of 321 pm. Figure 3 shows a top view onto the isoalloxazine plane of **4**; the bond lengths and angles are mostly in the rather normal range.

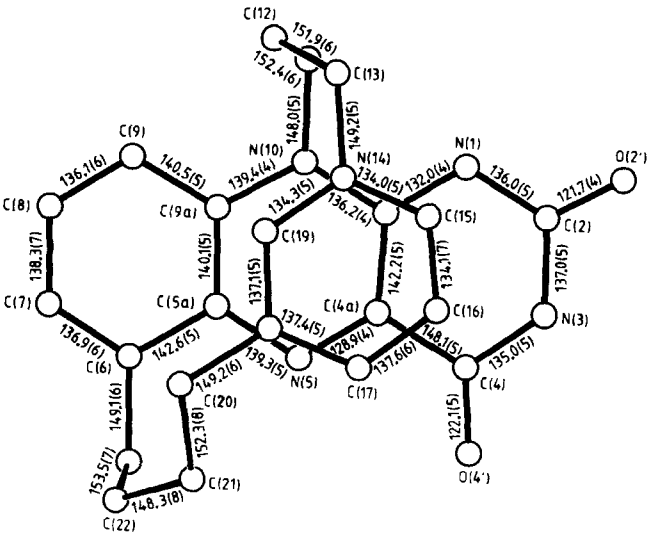

Figure 3. Molecular structure of the moiety **4** part of **4**-perchlorate in a top view onto the isoalloxazine plane

16-Methoxycarbonyl[4](5,1)pyridinio[3](10,6)isoalloxazinophane Iodide (**2, X = I**): From a solution of **2(X = I)** in methanol, containing traces (< 1%) of water, crystals of a monohydrate of **2(X = I)** were obtained which were suitable for an X-ray analysis. Bond lengths and bond angles within the isoalloxazine and nicotinic ester subunits do not show any significant deviations from expected values. The methoxycarbonyl group on C(16) is coplanar to the pyridinio ring, thus exerting the normal electron-withdrawing effect. The isoalloxazine unit deviates slightly from planarity due to the traction of the cyclophane bridges on C(6) and N(10). The angle of inclination between the least-squares planes of the pyridinio and isoalloxazino units amounts to 16.0(1)°. The average distance between the two subunits of **2** is ca. 350 pm, and the two redoxactive centers C(17) and N(5) are in a similar mutual contact as the corresponding atoms of the nicotinamide and the flavin units in glutathione reductase (Figure 4).

In the crystal the units of **2** form vertical stacks in which the nicotinic ester and isoalloxazine substructures alternate; due to the lateral shift between the units there is, however, no significant intermolecular overlap. As discussed for the preceding structures, there are lateral intermolecular interactions leading to a "dimerization", in this case by N(3)−H···O(2″) hydrogen bonds. The iodide ions are not positioned close to the positively charged pyridinium nitrogens nor do they show any other specific contacts of interest. The water molecules, too, with all distances to the closest neighboring atoms >324 pm obviously do not participate either essentially in intermolecular interactions.

16-Carboxylato[4](5,1)pyridinio[3](10,6)isoalloxazinophane (**34**): The zwitterionic **34** was crystallized by carefully overlayering an aqueous solution of **34** with acetone. The elementary unit contains, in addition of two molecules of

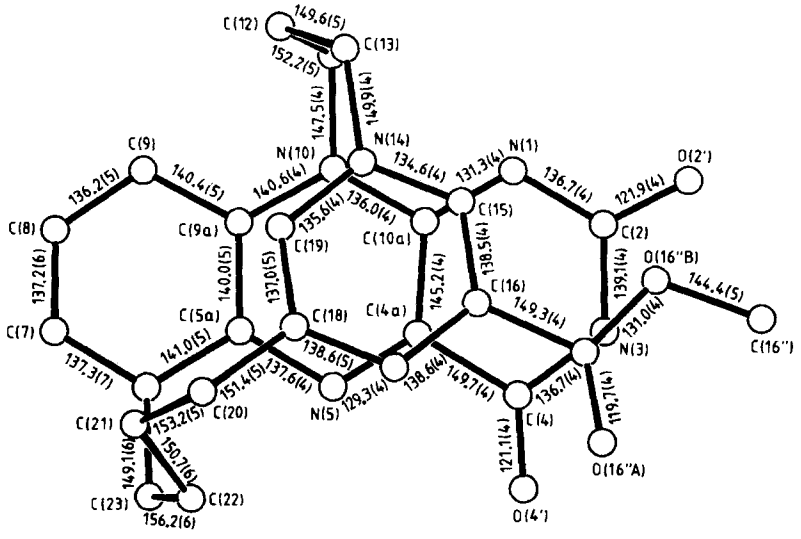

Figure 4. Molecular structure of the moiety **2** part of **2(X=I**, monohydrate) in a top view onto the isoalloxazine plane

34, four molecules of water. The bond lengths in the isoalloxazine part correspond closely to those in simple isoalloxazines although the isoalloxazine system in **34** deviates slightly from planarity like that of **2**; the bond lengths in the pyridinium unit, too, are normal. In **34** the two ring systems deviate more than in **2** and **4** from a parallel arrangement, the interplanar angle between the mean planes of the isoalloxazine and pyridinium substructures amounting to 19.8(1)°.

As was found for other isoalloxazinophanes as well (see above), in the crystal lattice two molecules of **34** "dimerize" laterally, in this case by two N(3)–H···O(4′) hydrogen bonds [d = 291.3(4) pm, ∢ about 166(4)°]. A unique feature of the crystal structure of **34** is the interaction between the carboxylate oxygen and the pyridinium nitrogen of the next vertical neighbor leading to a short distance of 302 pm between the corresponding atoms O(16″A) and N(14). This obviously electrostatic interaction explains that the carboxylate group is twisted out of the pyridinium plane by about 20°. In contrast to **2(X=ClO$_4$)**, in the crystal of **34** the water molecules participate in hydrogen bonds to the carboxylate oxygen O(16″B) and to the carbonyl oxygen O(2′). Figure 5 shows the molecular structure of **34** in a top view onto the mean plane of the isoalloxazine system.

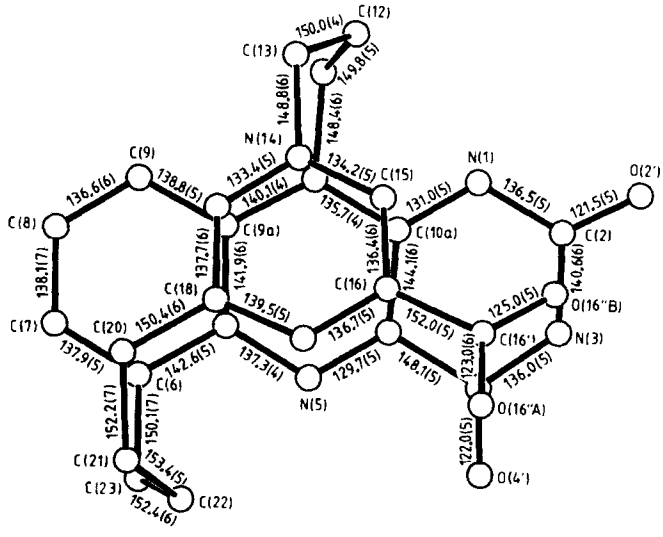

Figure 5. Molecular structure of **34** in a top view perpendicular to the mean plane of the isoalloxazine unit

Preliminary Results of Electron-Absorption Spectroscopy on Isoalloxazinophanes

Electron-absorption spectra of flavins (isoalloxazines) have been studied extensively in the past in connection with the unique biochemical importance of this group of compounds[24]. All the isoalloxazinophanes dealt with in this paper contain a dialkylisoalloxazine chromophore with the same substitution pattern as present in 6,10-dimethylisoalloxazine (**36**). The components linked to the isoalloxazine unit by the cyclophane skeleton are, however, electronically different: In **3** the *meta*-bridged benzene moiety is not expected to exert a perceivable electronic effect on the isoalloxazine unit; in the corresponding pyridiniophane **4** a moderate electron acceptor is neighboring the isoalloxazine, and in **2** the pyridinium system of the nicotinic ester unit represents further increased acceptor strength. A special case is the zwitterionic isoalloxazinophane **34** since the electron affinity of the carboxylatopyridinium unit is expected to increase with increasing protonation. Thus, the question was to what an extent these different structures in close and fixed neighborhood would interfere with the isoalloxazine chromophore by transannular interaction.

Table 1 lists the maxima and the molar extinction coefficients of the three characteristic flavin absorption bands above 250 nm for the isoalloxazinophanes **3**, **4(X=Cl)**, **2(X=Cl)**, and **34** together with the simple isoalloxazine **36**. Although the informative value of these data is limited because of the broadness and the lack of structure of these absorptions, it can be concluded that within the group of the four isoalloxazinophanes of different structure the close

agreement of wavelenghts and intensities and the similarities of solvent and *p*H dependences rule out specific transannular interactions of the charge-transfer type. On the other hand, there is obviously an unspecific cyclophane effect since the two first absorption bands of all isoalloxazinophanes are significantly red-shifted as compared to **36**.

Table 1. Absorption maxima and extinction coefficients of isoalloxazinophanes **3**, **4(X=Cl)**, **2(X=Cl)**, and **34** in comparison with 6,10-dimethylisoalloxazine (**36**)

Compound	λ_{max}[nm](ε 10^{-3} l mol^{-1} cm^{-1})			Solvent
3	269 (24.2)	359 (7.0)	444 (7.3)	acetonitrile
	269 (23.2)	368 (7.1)	443 (6.8)	methanol
	267 (25.5)	387 (9.2)	445 (6.4)	citrate buffer *p*H 3/10% methanol
	266 (24.7)	387 (9.2)	444 (6.5)	phosphate buffer *p*H 8/10% methanol
4(X=Cl)	268 (27.0)	359 (7.6)	441 (7.0)	acetonitrile
	264 (29.2)	368 (8.2)	440 (7.1)	methanol
	263 (31.8)	389 (10.5)	444 (6.6)	citrate buffer *p*H 4 (0.1 M)
	262 (30.0)	369 (10.3)	445 (6.6)	borate buffer *p*H 9 (0.1 M)
2(X=Cl)	264 (23.5)	359 (6.9)	443 (5.9)	acetonitrile
	264 (28.7)	369 (8.1)	441 (6.3)	methanol
	265 (25.5)	391 (9.3)	446 (5.7)	citrate buffer *p*H 3 (0.1 M)
	265 (25.1)	390 (9.0)	447 (5.8)	phosphate buffer *p*H 8 (0.1 M)
34	267	358	442	acetonitrile[a]
	266 (26.4)	366 (7.6)	441 (6.6)	methanol
	266 (25.6)	389 (9.0)	445 (5.7)	citrate buffer *p*H 3 (0.1 M)
	266 (25.0)	389 (8.7)	445 (5.6)	phosphate buffer *p*H 8 (0.1 M)
36	266 (31.2)	348 (7.9)	436 (8.8)	acetonitrile
	264 (32.4)	358 (8.2)	434 (8.5)	methanol
	262 (35.0)	378 (11.1)	433 (8.4)	citrate buffer *p*H 3 (0.1 M)
	262 (32.3)	378 (10.1)	433 (7.6)	phosphate buffer *p*H 8 (0.1 M)

[a]) Due to low solubility no correct ε values were obtained.

The attempt to increase the donor strength of the isoalloxazine units by selective reduction (irradiation under anaerobic conditions, presence of EDTA disodium salt[25]) resulted, however, in a different behavior of the isoalloxazinophanes: whereas for **3** no new absorptions at longer wavelengths occur, for **4(X=Cl)**, **2(X=Cl)** and **34** additional broad absorption bands extending beyond 700 nm are observed. These new absorptions most likely are to be assigned to transannular charge-transfer transitions between the reduced isoalloxazine as electron donor and the pyridinium system as acceptor.

Reduction with sodium dithionite is known to result in reductions of both the isoalloxazine and pyridinium systems[25]. In fact, when **2(X=Cl)** was reduced with sodium dithionite (methanol, anaerobic conditions) no long-wavelength absorption was observed. In this case, for a sample reduced in a ^{1}H-NMR probe with careful exclusion of oxygen an ^{1}H-NMR spectrum was obtained most signals of which could unambiguously be assigned (COSY, NOESY). This ^{1}H-NMR spectrum confirms the complete reduction to a cyclophane containing the reduced isoalloxazine as well as the 1,4-dihydropyridine system[27]. Further investigations of the redox behavior of these isoalloxazinophane systems are intended.

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Experimental

Melting points: Büchi SMP 20 and 512; Bock Monoskop M (m.p. >240°C); the given melting points are uncorrected. – UV/Vis-absorption spectra: Cary 2300 (Varian). – IR: Beckman IR-4240 (KBr), Perkin-Elmer FT-IR spectrometer 1760X (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 WP-80, HX-360, AM-500 (internal reference tetramethylsilane). – Microanalysis: Elemental Analyzer 1106 Carlo Erba. – Analytical TLC: DC Micro Cards Polygram SIL G/UV_{254} and Polygram ALOX N/UV_{254}, Macherey-Nagel. – CC: Silica gel Sili Tech 63–200 μm, ICN Biomedicals; alumina 90, activity II–III, 63–200 μm, Merck. – Flash chromatography: Silica gel 60, 35–70 μm, Amicon. – Preparative HPLC: Nucleosil (–NMe_2, 10 μm), Macherey-Nagel. – Gel permeation chromatography: Sephadex LH 20 and G 10, Pharmacia LKB Biotechnology; Biogel P2, Bio-Rad. – Ion exchange chromatography: for anion exchange weakly basic Sephadex DEAE A-25 (Pharmacia) and strongly basic DOWEX 1X8 (Serva); for cation exchange strongly acidic Sephadex SP C-25 (Pharmacia); elution buffer triethylammonium acetate was prepared by neutralization of 2 N aqueous acetic acid with triethylamine to pH 7.0.

3-Acetylamino-2-nitrobenzoic Acid: In a modified procedure in analogy to ref.[13] 326 g (1.82 mol) of 3-acetylaminobenzoic acid was added in small portions to 750 ml of precooled fuming nitric acid (d = 1.5 g cm^{-3}) at –5 to 0°C within a period of 2 h. The temperature was controlled by the addition of small pieces of dry ice to the reaction mixture. After stirring for 45 min, the mixture was poured on 1400 g of ice. The precipitate was sucked off, washed with ice water until the filtrate reached a pH of 2–3, and the crude mixture of isomers was dried at 50°C/40 mbar. For the separation of the isomers formed the product mixture was suspended in 1700 ml of water, and with vigorous stirring 200 g of barium hydroxide octahydrate was added. Then, after addition of 200 g of barium carbonate the reaction mixture was heated for 1 h to 90–100°C with stirring, and again 200 g of barium carbonate was added. After further 30 min the precipitate consisting of the barium salt of the isomeric acid and of barium carbonate was filtered off from the hot reaction mixture whereas the barium salt of the required 3-acetylamino-2-nitrobenzoic acid remained in solution and was precipitated from the filtrate by addition of half-concentrated hydrochloric acid. After 12 h the yellow precipitate was filtered off and dissolved in 2000 ml of boiling ethanol. From this solution after addition of the same volume of water on cooling the product precipitated and was filtered off after 16 h. By evaporating the ethanol from the filtrate, further product was obtained which was recrystallized as mentioned before; yield: 114 mg (28%), m.p. 235°C (dec.). – Repetition of the separation of the isomeric acids via their barium salts led to a purer, isomer-free product: fine yellow needles, m.p. 243°C (dec.). – MS: *m/z* (%) = 224 (12, M^+), 192 (14), 182 (100), 177 (23). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ = 2.03 (s, 3H, $COCH_3$), 7.67 ("t", $J \approx$ 7.7 Hz, 1H, 5-H), 7.73–7.77 (m, 2H, 4,6-H), 9.93 (br. s, 1H, NH), 13.86 (br. s, 1H, COOH). – $C_9H_8N_2O_5$ (224.17): calcd. C 48.22, H 3.60, N 12.50; found C 48.35, H 3.60, N 12.62.

3-Acetylamino-2-nitrobenzoyl Chloride: A suspension of 100.8 g (0.45 mol) of 3-acetylamino-2-nitrobenzoic acid in 400 ml of thionyl chloride after addition of 1 ml of dimethyl formamide was stirred for 30 min at 20°C, then for 15 min at 60°C, and was eventually heated to reflux until a transparent solution was obtained. Thionyl chloride was distilled off in vacuo, and the remaining product was extracted with 400 ml and then again with 200 ml of hot tetrachloromethane. The light yellow product (79 g, yield 73%) was directly used for the following reaction. For analysis the product was crystallized from tetrachloromethane (m.p. 140–141°C) and toluene (m.p. 147.5°C). – MS: *m/z* (%) = 242 (12, M^+), 207 (14), 200 (29), 165 (100). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ = 2.27 (s, 3H), 7.53 (dd, J = 8.0 and 1.1 Hz, 1H), 7.68 ("t", $J \approx$ 8.0 Hz, 1H), 8.72 (d, J = 8.0 Hz, 1H), 9.07 (br. s, 1H). – $C_9H_7ClN_2O_4$ (242.62): calcd. C 44.56, H 2.91, Cl 14.61, N 11.55; found C 44.56, H 2.71, Cl 14.48, N 11.40.

3-Acetylamino-2-nitrobenzyl Alcohol (**19**). – a) *By Reduction of 3-Acetylamino-2-nitrobenzoyl Chloride:* A solution of 87.1 g (0.36 mol) of the aforementioned benzoyl chloride in 700 ml of dry tetrahydrofuran was added under argon at 10°C to a suspension of 17.1 g (0.45 mol) of sodium borohydride in 360 ml of dry tetrahydrofuran and 170 ml of dry DMF. After stirring at room temp. for 2 h and heating at reflux for 1 h, 500 ml of a saturated aqueous ammonium chloride solution was carefully added. Eightfold extraction with 200 ml each of diethyl ether, drying of the combined ether extracts with magnesium sulfate and evaporation of the solvent in vacuo yielded a yellow solid which was crystallized from water; yield of **19**: 46.7 g (62%) of light yellow crystals, m.p. 164–165°C. – MS: *m/z* (%) = 210 (70, M^+), 168 (65), 151 (100), 133 (35), 105 (60). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ =

1.99 (s, 3H), 4.53 (d, J = 5.6 Hz, 2H), 5.46 (t, J = 5.6 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.56 ("t", $J \approx$ 7.8 Hz, 1H), 9.94 (br. s, 1H, NH). – $C_8H_{10}N_2O_4$ (210.19): calcd. C 51.43, H 4.80, N 13.33; found C 51.16, H 4.95, N 13.17.

b) *By the Mixed-Anhydride Method from 3-Acetylamino-2-nitrobenzoic Acid:* To a suspension of 4.48 g (20 mmol) of 3-acetylamino-2-nitrobenzoic acid in 30 ml of dry tetrahydrofuran 2.8 ml (20 mmol) of triethylamine was added. After stirring for 5 min in an ice bath 2.0 ml (21 mmol) of ethyl chloroformate in 5 ml of dry tetrahydrofuran was carefully added within 10 min; the reaction mixture was stirred for further 30 min. Then the white precipitate was filtered off and washed with 5 ml of tetrahydrofuran. The combined filtrate was added within 30 min to a freshly prepared, ice-cold solution of 1.8 g (50 mmol) of sodium borohydride in 20 ml of water at 10–15°C. After stirring for 4 h at room temp. 100 ml of a saturated aqueous ammonium chloride solution was added. Tenfold extraction with 50 ml each of diethyl ether after drying, evaporation of the solvent from the combined extracts, filtration of the residue from ethyl acetate/ethanol (1:1) through silica gel, distillation of the solvents and crystallization from water resulted in 3.14 g (yield 75%) of 3-acetylamino-2-nitrobenzyl alcohol, which was identical with the product obtained by method a).

3-Acetylamino-2-nitrobenzaldehyde (**6**): A suspension of 17.2 g (82 mmol) of 3-acetylamino-2-nitrobenzyl alcohol, 8.0 g of magnesium sulfate, 10.0 g of Florisil and 26.45 g (123 mmol) of pyridinium chlorochromate in 200 ml of dichloromethane was stirred for 7 h at 20°C. The solution was decanted from the precipitate which was extracted several times with dichloromethane. The combined organic extracts were filtered from dichloromethane through a short column of neutral alumina; from the filtrate the solvent was evaporated, and the residue was crystallized from water; yield: 9.5 g (56%) of **6**, yellow needles of m.p. 146–147°C. – MS: *m/z* (%) = 208 (28, M^+), 178 (34), 166 (100), 149 (22), 119 (26). – ^{1}H NMR (80 MHz, $CDCl_3$): δ = 2.27 (s, 3H), 7.5–7.8 (m, 2H), 8.67 (dd, J = 7.1 and 2.8 Hz, 1H), 8.96 (br. s, 1H), 10.03 (s, 1H). – $C_9H_8N_2O_4$ (208.17): calcd. C 51.93, H 3.87, N 13.46; found C 52.00, H 4.00, N 13.42.

1-(3-Bromopropyl)-3-(3-triphenylphosphoniopropyl)benzene Bromide (**7**): From a mixture of 24.0 (75.0 mmol) of 1,3-bis(3-bromopropyl)benzene[15], 50 ml of benzene and 300 ml of methylcyclohexane ca. 130 ml of solvent was distilled off to remove traces of water. To the remaining solution 13.1 g (50 mmol) of triphenylphosphane was added with exclusion of moisture, and the solution was stirred for 160 h at 110–115°C. After cooling 100 ml of diethyl ether was added; the resulting precipitate was sucked off, three times washed with 50 ml of diethyl ether each, and dissolved in 100 ml of dichloromethane. After filtration and dropwise addition of about 70 ml of diethyl ether to the filtrate until beginning turbidity, the solution was kept at +2°C for 12 h; the precipitate formed (mainly the corresponding bisphosphonium salt) was filtered off, and to the filtrate additional 200 ml of diethyl ether was added. The precipitate formed after 15 h at +2°C was sucked off and dissolved in 60 ml of dichloromethane. To this solution 110 ml of ethyl acetate was added at 20°C with stirring. The white crystals formed after 12 h at +2°C were sucked off, washed with diethyl ether and dried over silica gel; yield: 18.3 g (63%) of **7** as microcrystalline colorless needles of m.p. 187–188°C. – ^{1}H NMR (80 MHz, $CDCl_3$): δ = 1.70–2.30 (m, 4H, $-CH_2-CH_2-CH_2-$), 2.72 (t, J = 7.0 Hz, 2H, ar-CH_2), 3.00 (t, J = 7.0 Hz, 2H, ar-CH_2), 3.35 (t, J = 7.0 Hz, 2H, CH_2Br), 3.60–4.00 (m, 2H, $CH_2\overset{\oplus}{P}Ph_3$), 6.90–7.10 (m, 4H, ar-H), 7.70–7.90 (m, 15H, PPh_3). – $C_{30}H_{31}Br_2P$ (582.37): calcd. C 61.87, H 5.37, Br 27.44; found C 61.95, H 5.33, Br 27.48.

(Z)- and (E)-1-(3-Acetylamino-2-nitrophenyl)-4-[3-(3-bromopropyl)-phenyl]butene-1 (**8** *and* **9**): To a suspension of 66.0 g (113 mmol) of **7** in 145 ml of dry THF a solution of 13.97 g (125 mmol) of freshly sublimated potassium *tert*-butylate in 170 ml of THF was added dropwise at –78°C under argon. After stirring for 30 min at –78°C a solution of 23.6 g (113 mmol) of **6** in 225 ml of THF was added over 15 min, and the solution was stirred for 90 min at –78°C. Then additional 2.8 g of potassium *tert*-butylate in 34 ml of THF was added, and the reaction mixture was allowed to warm up to room temp. within 1 h. After stirring for 2 h the solution was poured into a mixture of 120 ml of a saturated aqueous ammonium chloride solution, 50 ml of a saturated sodium chloride solution and 250 ml of diethyl ether. The ethereal phase was separated, and the aqueous phases were subsequently washed with 150 ml each of a saturated aqueous sodium chloride solution, an aqueous sodium hydrogen sulfite solution, a sodium hydrogen carbonate solution and again twice with a saturated sodium chloride solution. After drying with anhydrous magnesium sulfate the solvent was distilled off in a rotary evaporator, and the crude product (71 g of brown oil) was chromatographed from dichloromethane/diethyl ether/toluene (75:20:5) on a silica gel column (h = 140 cm, d = 15 cm). The fraction with $R_f \approx$ 0.44 contained 20.58 g of the pure Z isomer **8**, the fraction with $R_f \approx$ 0.40 yielded 6.80 g of the pure E isomer **9**, and the intermediate fraction (6.14 g) consisted of a mixture (about 1:1) of the two isomers: overall yield 33.52 g (68%) of a mixture of **8** and **9** in a ratio of ca. 70:30.

8: ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.10–2.17 (m, 2H, $-CH_2-CH_2Br$), 2.19 (s, 3H, $-COCH_3$), 2.33–2.40 (m, 2H, 3-CH_2), 2.66 and 2.73 (each t, J = 7.4 Hz, together 4H, 4-CH_2 and $-CH_2-CH_2-CH_2Br$), 3.37 (t, J = 6.5 Hz, 2H, $-CH_2Br$), 5.83 (dd, J = 7.8 and 11.7 Hz, 1H, $-CH_2-CH=CH-$), 6.44 (d, J = 11.7 Hz, 1H, $-CH_2-CH=CH-$), 6.85 (d, J = 7.8 Hz, 1H, ar-H), 6.95–6.97 (m, 2H, ar-H), 7.02 (d, J = 7.8 Hz, 1H, ar-H), 7.16–7.21 (m, 1H, ar-H), 7.38–7.42 (m, 1H, ar-H), 8.16 (d, J = 7.3 Hz, 1H, 6-H of NO_2-subst. benzene ring), 8.47 (br. s, 1H, NH). – $C_{21}H_{23}BrN_2O_3$ (431.34): calcd. C 58.48, H 5.37, N 6.49, Br 18.53; found C 58.67, H 5.65, N 6.30, Br 18.49.

9: ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.11–2.19 (m, 2H, $-CH_2-CH_2Br$), 2.19 (s, 3H, $-COCH_3$), 2.51–2.58 (m, 2H, 3-CH_2), 2.73–2.81 (m, 4H, 4-CH_2 and $-CH_2-CH_2-CH_2Br$), 3.38 (t, J = 6.5 Hz, 2H, $-CH_2Br$), 6.20–6.30 (m, 1H, $-CH_2-CH=CH-$), 6.43 (d, J = 15.6 Hz, 1H, $-CH_2-CH=CH-$), 7.03–7.06 (m, 3H, ar-H), 7.20–7.30 (m, 2H, ar-H), 7.40–7.45 (m, 1H, ar-H), 8.10 (d, J = 7.8 Hz, 1H, 6-H of NO_2-subst. benzene ring), 8.25 (br. s, 1H, NH). – $C_{21}H_{23}BrN_2O_3$ (431.34): calcd. C 58.48, H 5.37, N 6.49, Br 18.53; found C 58.85, H 5.51, N 6.27, Br 18.66.

(Z)-1-Acetyl-20-nitro-1-aza[4.4]metacyclophan-13-ene (**10**): A solution of 10.0 g (23.2 mmol) of the aforementioned Z isomer **8** in 2 l of toluene was dropped within 36 h to a refluxing mixture of 800 ml of toluene, 400 ml of a 2.5% aqueous sodium hydroxide solution and 5 g (15.5 mmol) of tetra-*n*-butylammonium bromide. After refluxing for further 2 h, the aqueous layer was separated and extracted with 150 ml of toluene. The combined organic phases were washed with a saturated aqueous ammonium chloride solution and water and subsequently dried with anhydrous magnesium sulfate. Distilling off the solvent in a rotary evaporator left 8.28 g of a yellow solid which was triturated with 20 ml of diethyl ether and the mixture was sucked off after standing for 12 h at –18°C; yield: 6.78 g (83%) of **10** as a bright yellow crystal powder of m.p. 160–161.5°C. From the mother liquor the solvent was distilled off and the residue treated with 4 ml of diethyl ether to afford additional 140 mg of **10**. – MS: *m/z* (%) = 350 (3, M^+), 322 (21), 292 (30), 291 (100), 263 (25), 173 (38), 161 (26), 145 (48), 132 (30), 131 (65), 117 (63), 115 (45), 105 (45) a.o. – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.14–1.30 (m, 1H), 1.86–1.97 (m, 1H), 2.01 (s, 3H, $COCH_3$), 2.20–2.40 (m, 2H), 2.43–2.72 (m, 4H), 2.78–2.90 (m, 1H), 4.44–4.56 (m, 1H, 2-H), 5.83–5.95 (m, 1H, 13-H), 6.15 (d, J = 11.7 Hz, 1H, 14-H), 6.26 (s, 1H, ar-10-H), 6.81 ("d", $J \approx$ 7.5 Hz, 1H, ar-6- or ar-8-H), 6.96 ("d", $J \approx$ 7.5 Hz, 1H, ar-8- or ar-6-H), 7.11–7.20 (m, 2H, ar-7- and ar-18-H), 7.29 ("d", $J \approx$ 7.5 Hz, 1H, ar-16-H), 7.50–7.54 (m, 1H, ar-17-H). – $C_{21}H_{22}N_2O_3$ (350.42): calcd. C 71.98, H 6.38, N 7.99; found C 72.23, H 6.56, N 7.93.

1-Acetyl-20-amino-1-aza[4.4]metacyclophane: 100 mg (0.285 mmol) of **10** dissolved in 5 ml of ethanol and 4 ml of glacial acetic acid in the presence of 10 mg of palladium catalyst (10% on charcoal) was hydrogenated at 20°C/760 Torr (2 h). After crystallization from methanol by addition of water the hydrogenation product was obtained as a colorless crystal powder of m.p. 203–204°C; 70 mg (76%). – MS: *m/z* (%) = 322 (100, M^+), 307 (25), 305 (52), 279 (22). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.30–1.53 (m, 3H), 1.58–1.75 (m, 2H), 1.83–1.95 (m, 1H), 1.94 (s, 3H, CH_3CO), 2.29–2.48 (m, 3H), 2.59–2.83 (m, 4H), 3.41 (br. s, 2H, NH_2), 4.18–4.27 (m, 1H, 2-H), 6.07 ("s", 1H, 10-H), 6.69–6.74 (m, 1H, ar-H), 6.85–6.90 (m, 3H, ar-H), 7.01 ("d", $J \approx$ 7.5 Hz, 1H, ar-H), 7.05–7.10 (m, 1H, ar-H). – $C_{21}H_{26}N_2O$ (322.45): calcd. C 78.22, H 8.13, N 8.69; found C 78.20, H 8.35, N 8.59.

(E,E)-1,21-Diacetyl-20,40-dinitro-1,21-diaza[4.4.4.4]metacyclophan-13,33-diene (**11**): In analogy to the preparation of **10** a solution of 2.0 g (4.6 mmol) of **9**, the E isomer of **8**, in 250 ml of toluene was dropped within 5 h to a boiling mixture of 250 ml of toluene, 140 ml of a 2.5% aqueous sodium hydroxide solution and 1.0 g (3.1 mmol) of tetra-*n*-butylammonium bromide. After heating at reflux for 24 h the aqueous phase was separated from the cooled reaction mixture and extracted twice with 100 ml each of dichloromethane. The combined organic phases were washed with 100 ml each of a saturated ammonium chloride solution and water, and dried with anhydrous magnesium sulfate. The residue, obtained by distilling off the solvent in a rotary evaporator, was treated with 5 ml of diethyl ether and kept for 15 h at –18°C. The precipitate was sucked off, washed with methanol and diethyl ether; yield: 570 mg (35%) of **11** which for analysis was recrystallized from DMF and dried at 150°C/10^{-3} Torr for 24 h. – MS: *m/z* (%) = 700 (4, M^+), 654 (54), 652 (20), 641 (24), 623 (64), 173 (36), 171 (30), 169 (22), 161 (28), 157 (48), 147 (38), 131 (100). – $C_{42}H_{44}N_4O_6$ (700.84): calcd. C 71.98, H 6.33, N 7.99; found C 71.82, H 6.10, N 8.03.

(Z)-20-Nitro-1-aza[4.4]metacyclophan-13-ene (**12**): A solution of 6.0 g (17.1 mmol) of **10** in 450 ml of ethanol after addition of 108 g of potassium hydroxide and 72 ml of water was heated under argon to 85–90°C. The deacetylation was controlled by TLC [silica gel, dichloromethane/diethyl ether (85:15); **10**: $R_f \approx$ 0.40; **12**: $R_f \approx$ 0.64]. After about 70 min 100 ml of cyclohexane was added; heating was continued for additional 15 min with stirring. Then the reaction mixture was cooled to room temp. and, after

addition of 250 ml of water, extracted five times with 150 ml each of diethyl ether. The combined extracts were washed with 100 ml of a saturated aqueous ammonium chloride and 100 ml of water. After drying with anhydrous magnesium sulfate and evaporation of the solvent, **12** was obtained by CC (h = 18 cm, d = 8 cm; silica gel; trichloromethane/cyclohexane, 9:1) and recrystallization from *n*-hexane; yield: 4.17 g (79%) of **12** as small coral-red needles, m.p. 118.5–119.5°C. – MS: *m/z* (%) = 308 (100, M^+), 291 (22), 280 (45), 279 (40), 263 (55), 261 (30), 163 (23), 151 (70), 147 (77), 145 (75), 131 (72), 119 (50), 117 (62), 115 (28), 105 (50), 103 (20), 91 (38). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.93–2.03 (m, 1H), 2.13–2.30 (m, 4H), 2.59–2.68 (m, 3H), 3.39–3.46 (m, 1H, 2-H), 3.80–3.87 (m, 1H, 2-H), 5.63–5.71 (m, 1H, 13-H), 6.30–6.33 (m, 2H, 10,14-H), 6.78–7.06 (m, 5H, 6,7,8,16,18-H), 7.18–7.22 (m, 1H, 17-H), 7.30–7.50 (br. s, 1H, NH). – $C_{19}H_{20}N_2O_2$ (308.38): calcd. C 74.00, H 6.54, N 9.0; found C 74.01, H 6.70, N 9.10.

[4]Metacyclo[3](10,6)isoalloxazinophane (**3**): For the catalytic hydrogenation of **12** to **5**, 800 mg (2.59 mmol) of **12** together with 800 mg (13.0 mmol) of boric acid were dissolved in 80 ml of acetic acid. In the presence of 150 mg of palladium catalyst (10% on charcoal) the hydrogenation at 20°C/760 Torr was continued until about 270 ml of hydrogen had been taken up (theoretical uptake for hydrogenation of double bond and nitro group 249 ml). Due to its oxidation sensitivity the hydrogenation product **5** was not isolated but the solution of the hydrogenation reaction was filtered off from the catalyst by means of a reversing frit by which the reaction mixture was directly added to a solution of 1.39 g (6.5 mmol) of alloxan hydrate and 1.50 g (24.3 mmol) of boric acid in 100 ml of glacial acetic acid. Under argon and with the exclusion of light the reaction mixture was kept at 55–60°C with stirring for 14 h. During this process the originally colorless solution changed to an intense yellow-green color and became strongly fluorescent in UV light. The solvent was distilled off in vacuo, and the boric acid was removed by threefold distillation of 100 ml each of methanol into a rotary evaporator. The remaining residue was dissolved in trichloromethane/methanol (1:1), the solution filtrated and adsorbed on 6 g of Florisil. By chromatography (silica gel; h = 40 cm, d = 5 cm; trichloromethane/methanol, 19:1) from the intensively fluorescing fraction of $R_f \approx 0.37$ after removal of the solvents and crystallization from acetic acid/diethyl ether 710 mg (71%) of **3** was obtained as a yolk-yellow crystal powder, m.p. 293–296°C (dec.). – MS: *m/z* (%) = 386 (100, M^+), 255 (18), 242 (24), 229 (22), 228 (18). – ^{1}H NMR (360 MHz, $CDCl_3$; assignments by decoupling and NOE experiments): δ = 0.88–1.01 (m, 1H), 1.69–1.87 (m, 2H), 1.88–2.00 (m, 1H), 2.02–2.13 (m, 1H), 2.15–2.26 (m, 1H), 2.30–2.43 (m, 1H), 2.52–2.64 (m, 3H), 2.81–2.90 (m, 1H), 3.77–3.86 (m, 1H), 4.34–4.44 (m, 1H, 11-H), 5.64–5.74 (m, 2H, 11,19-H), 6.70 ("d", $J \approx 7.0$ Hz, 1H, 15-H), 6.82–6.90 (m, 2H, 16,17-H), 7.38 ("d", $J \approx 7.1$ Hz, 1H, 7-H), 7.61 ("d", $J \approx 8.5$ Hz, 1H, 9-H), 7.72–7.79 (m, 1H, 8-H), 8.41 (br. s, 1H, 3-H). – X-Ray analysis: See above and Table 2. – $C_{23}H_{22}N_4O_2$ (386.45): calcd. C 71.48, H 5.74, N 14.50; found C 71.24, H 5.75, N 14.51.

3-(3-Triphenylphosphoniopropyl)pyridine Bromide: A mixture of 49.45 g (360 mmol) of 3-(3-hydroxypropyl)pyridine, 94.4 g (360 mmol) of triphenylphosphane and 130 ml of 48% hydrobromic acid was heated with stirring for 1 h at 135°C; then 60 ml of toluene was added, and the solution was heated at reflux in a water separator until no more water distilled off (ca. 3 h). After addition of further 40 ml of 48% hydrobromic acid the reaction mixture was refluxed again on a water separator for 6 h. To the residue 100 ml of water was added, and the suspension was digested four times with 100 ml each of diethyl ether. The aqueous solution was neutralized by addition of 2 N aqueous sodium hydroxide and concd. ammonia until a pH of 6 to 7 was reached. The resulting suspension of the phosphonium salt was washed four times with 100 ml each of diethyl ether until the extracts were free from triphenylphosphane. After 12 h at 2°C the product was sucked off, washed with water and diethyl ether and dried at 100°C in vacuo; yield: 139 g (84%) of a colourless powder of m.p. 202–204°C (crystallized from water). – ^{1}H NMR (80 MHz, $CDCl_3$): δ = 1.75–2.25 (m, 2H, $-CH_2-CH_2-CH_2-$), 3.12 (t, J = 7.5 Hz, 2H, pyr-CH_2-), 3.75–4.18 (m, 2H, $-CH_2PPh_3$), 7.19 (dd, J = 4.8 and 7.8 Hz, 1H, pyr-5-H), 7.55–8.01 (m, 16H, pyr-4-H and $-PPh_3$), 8.36–8.44 (m, 2H, pyr-2,6-H). – $C_{26}H_{25}BrNP$ (462.37): calcd. 67.54, H 5.45, Br 17.28, N 3.03, P 6.70; found C 67.55, H 5.35, Br 17.27, N 3.12, P 6.73.

(Z)- and (E)-1-(3-Acetylamino-2-nitrophenyl)-4-(3-pyridyl)butene-1 (**13**/**14**): To a suspension of 85.0 g (184 mmol) of the aforementioned phosphonium salt and 3.00 g (11.3 mmol) of [18]crown-6 in 150 ml of dry THF a solution of 31.8 g (283 mmol) of freshly sublimated potassium *tert*-butylate in 150 ml of dry THF was added at 0–10°C within 20 min with careful exclusion of oxygen and moisture. After stirring at 0°C for 30 min the dark red ylide solution was cooled to −78°C, and a solution of 20.0 g (96.1 mmol) of aldehyde **6** in 220 ml of THF was added. The mixture was stirred at −78°C for 30 min and then allowed to warm up to 0–10°C within 45 min. After stirring for 2 h at this temperature, 150 ml of citrate buffer (pH 4) was added; by the addition of 2 N hydrochloric acid the pH of the reaction mixture was adjusted to 4 to 5 and extracted with ca. 400 ml of ethyl acetate. The organic extract was washed twice with 50 ml each of water, and then the basic products were extracted three times with 100 ml each of 2 N hydrochloric acid. After washing the combined acid extracts with 100 ml of diethyl ether and 100 ml of dichloromethane, addition of 2 N aqueous sodium hydroxide liberated the pyridine-containing products which were extracted three times with 150 ml each of dichloromethane. These combined extracts were washed with 100 ml each of water and of concd. sodium chloride solution. After drying with anhydrous magnesium sulfate the solvent was evaporated, and the oily residue was adsorbed on Florisil and chromatographed on silica gel with ethyl acetate/ethanol (95:5). From the eluate following an intensely yellow first fraction the solvent was evaporated, and the product was crystallized from ethyl acetate/diethyl ether, sucked off, washed with *n*-hexane and dried in vacuo at 70° to yield 28.1 g (94%) of a mixture of (*Z*)-**13** and (*E*)-**14** as a microcrystalline powder of m.p. 112–120°C. – MS: *m/z* (%) = 311 (100, M^+), 296 (85), 267 (25), 265 (20), 252 (20), 177 (60), 131 (50), 130 (25), 107 (45), 106 (45), 93 (50), 92 (40). – $C_{17}H_{17}N_3O_3$ (311.34): calcd. C 65.58, H 5.50, N 13.50; found C 65.81, H 5.69, N 13.52.

By chromatography on silica gel (eluent: ethyl acetate) partial separation of 2.0 g of the **13**/**14** mixture into **13** and **14** was achieved: **A** ($R_f \approx 0.30$): 149 mg; m.p. 167–170°C (from diethyl ether) **14** plus ca. 10% **13** (assignment by ^{1}H NMR, see below); **B** (intermediate fraction, $R_f \approx 0.23$–0.29): 992 mg; **C** ($R_f \approx 0.24$): 480 mg; m.p. 117–119°C (from diethyl ether) **13** plus ca. 10% **14** (assignment by ^{1}H NMR).

Isomer **13**: ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.21 (s, 3H, CH_3CO-), 2.34–2.41 (m, 2H, $-CH=CH-CH_2-CH_2-$), 2.70 (t, J = 7.5 Hz, 2H, $-CH=CH-CH_2-CH_2-$), 5.81 (td, J = 7.5 and 11.5 Hz, 1H, $-CH=CH-CH_2-CH_2-$), 6.49 (d, J = 11.5 Hz, 1H, $-CH=CH-CH_2-CH_2-$), 6.86 ("d", $J \approx 7.7$ Hz, 1H, ar-4-H), 7.21 (dd, J = 4.9 and 7.8 Hz, 1H, pyr-5-H), 7.41–7.46 (2 m, 2H, ar-5-H and pyr-4-H), 8.20 ("d", $J \approx 8.3$ Hz, 1H, ar-6-H), 8.39 (br. s, 1H, pyr-2-H), 8.44–8.46 (m, 1H, pyr-6-H), 8.48 (br. s, 1H, H/D exchange, NH).

Isomer **14**: ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.19 (s, 3H, CH_3CO-), 2.53–2.59 (m, 2H, $-CH=CH-CH_2-CH_2-$), 2.81 (t, $J \approx 7.5$, 2H, $-CH=CH-CH_2-CH_2-$), 6.20 (td, J = 6.9 and 15.6 Hz, 1H, $-CH=CH-CH_2-CH_2-$), 6.43 (d, $J \approx 15.6$ Hz, 1H, $-CH=CH-$

Table 2. Crystal and data collection parameters of **30**, **3**, **4(X=ClO$_4$)**, **2(X=I)**, and **34**

	30	3	4(X=ClO$_4$)	2(X=I)	34
Formula	$C_{21}H_{23}N_4O_4^+$ Br^-H_2O	$C_{23}H_{22}N_4O_2$	$C_{22}H_{22}N_5O_2^+$ $ClO_4^-3H_2O$	$C_{24}H_{24}N_5O_4^+$ I^-H_2O	$C_{23}H_{21}N_5O_4$ $2H_2O$
Molecular mass	493.35	386.45	541.95	591.41	467.49
a[pm]	842.1 (2)	1239.5 (1)	824.0 (2)	904.5 (1)	823.1 (2)
b[pm]	850.4 (3)	898.5 (1)	1081.0 (2)	1417.4 (2)	1042.4 (2)
c[pm]	1679.7 (4)	1804.8 (2)	1420.2 (2)	1883.7 (4)	1402.3 (2)
α[°]	94.08 (3)	--	80.91 (2)	--	108.98 (1)
β[°]	100.94 (3)	105.72 (2)	89.11 (2)	93.15 (2)	94.13 (1)
γ[°]	109.28 (3)	--	80.27 (2)	--	106.87 (1)
Space group	$P1$[a]	$P2_1/c$[b]	$P1$[a]	$P2_1/c$[b]	$P1$[a]
Z	2	4	2	4	2
Dx [g·cm^{-3}]	1.485	1.327	1.462	1.629	1.451
Max.sin Θ/λ [nm^{-1}]	6.6	6.8	6.2	6.2	6.2
No. of refelctions measured	4681	5122	4827	4726	4189
No. of reflections with I>3σ(I)	3030	3210	3037	3683	2428
R	0.051	0.040	0.065	0.035	0.059
Crystal size [nm]	0.3x0.2x0.2	0.3x0.3x0.15	0.4x0.3x0.1	0.3x0.1x0.1	0.25x0.20x0.15
Crystallized from	water	acetonitrile	water	methanol/ 1% water	water/acetone

[a] No. 2 Internat. Tables - [b] No. 14 Internat. Tables.

CH_2-CH_2-), 7.22–7.26 (m, 2H, pyr-5-H and ar-4-H), 7.41–7.46 (m, 1H, ar-5-H), 7.53 ("d", $J \approx 7.8$ Hz, 1H, pyr-4-H), 8.12 (br. "d", $J \approx 7.8$ Hz, 1H, ar-6-H), 8.30 (br. s, 1H, H/D exchange, NH), 8.47–8.51 (m, 2H, pyr-2,6-H).

(Z)- and (E)-1-[3-[Acetyl(3-chloropropyl)amino]-2-nitrophenyl]-4-(3-pyridyl)butene-1 (**15/16**): To a suspension of 7.91 g (120 mmol) of pulverized potassium hydroxide (85% KOH) in 75 ml of dry DMSO, which was stirred under argon for 10 min, 9.34 g (30.0 mmol) of **13/14** (≈9:1) was added. After stirring at room temp. for 5 min the reaction mixture was cooled in an ice bath until beginning solidification; then 28.3 g (180 mmol) of 1-bromo-3-chloropropane was added within 8 min. With ice cooling the reaction was stirred for 10 min and after warming up to room temp. the reaction mixture was poured onto a mixture of 250 g of crushed ice, 150 ml of a saturated aqueous ammonium chloride solution and 150 ml of dichloromethane. The organic layer was separated, and the aqueous phase was extracted three times with 80 ml each of dichloromethane. The combined organic phases were washed with 100 ml each of water and sodium chloride solution, dried with anhydrous sodium sulfate and concentrated in a rotary evaporator at 30–35°C. Chromatography of the crude product on silica gel (h = 20 cm, d = 8 cm; ethyl acetate, $R_f \approx 0.24-0.30$) yielded 9.92 g (85%) of the isomer mixture of **15** and **16** (ratio about 9:1) as a yellow viscous liquid, n^{20} = 1.5894. – MS: *m/z* (%) = 387 (4, M^+), 340 (100), 295 (40), 281 (92), 278 (26), 276 (36), 267 (26), 252 (38), 236 (35), 203 (26), 177 (42), 167 (68), 161 (32), 131 (64), a.o. – ^{1}H NMR (360 MHz, $CDCl_3$, only signals of **15** are listed): δ = 1.86 (s, 3H, CH_3CO-), 1.95–2.12 (m, 2H, $-CH_2-CH_2Cl$), 2.41–2.51 (m, 2H, $-CH=CH-CH_2-CH_2-$), 2.74 (t, J = 7.4 Hz, 2H, $-CH=CH-CH_2-CH_2-$), 3.30–3.43 (m, 1H, $N-CH_2-$), 3.53 (br. "t", $J \approx$ 7 Hz, 2H, $-CH_2Cl$), 3.95–4.06 (m, 1H, $N-CH_2-$), 5.88–5.97 (m, 1H, $-CH=CH-CH_2-CH_2-$), 6.36 (d, J = 11.7 Hz, 1H, $-CH=CH-CH_2-CH_2-$), 7.14–7.26 (m, 3H, ar-4,6-H and pyr-5-H), 7.46 ("d", $J \approx 7.7$ Hz, 1H, pyr-4-H), 7.50–7.55 (m, 1H, ar-5-H), 8.40–8.47 (m, 2H, pyr-2,6-H). – $C_{20}H_{22}ClN_3O_3$ (387.87): calcd. C 61.93, H 5.72, N 10.83; found C 62.23, H 5.61, N 10.86.

4-Acetyl-10-nitro-4-aza[4]metacyclo[4](3,1)pyridiniophan-11-ene Iodide (**17,X = I**) *and Chloride* (**17,X = Cl**): For the cyclization of **15** to **17** by Cl/I exchange and intramolecular *N*-alkylation of the pyridine unit, 9.92 g (25.6 mmol) of **15** (content of *E* isomer **16** about 10%) in 1000 ml of DMF in a high-dilution apparatus was added under argon over 17 h to a boiling solution of 42.5 g (256 mmol) of potassium iodide in 900 ml of DMF and 400 ml of acetone at a temperature rising from 92 to 110°C. The solvents were evaporated in vacuo, and the residue was extracted five times with 500 ml each of hot trichloromethane. The combined extracts were separated from inorganic salts by filtration, and from the filtrate the solvent was evaporated. The solution of the residue in 500 ml of water and 100 ml of methanol was washed three times with 100 ml each of diethyl ether and once with 100 ml of trichloromethane, filtrated, and the solvents were distilled off in vacuo. The solid product obtained was crystallized from methanol by addition of diethyl ether at –18°C: 5.20 g (42%; about 47% yield referred to the *Z* isomer **15**) of **17(X = I)** as white crystals of m.p. 250–251°C (dec.); $R_f \approx$ 0.21 (silica gel, ethanol/1 M aqueous ammonium acetate solution, 7:3). – $C_{20}H_{22}IN_3O_3$ (479.32): calcd. C 50.12, H 4.63, N 8.77; found C 50.09, H 4.46, N 8.54.

For further characterization **17(X=I)** was dissolved in water with addition of a small amount of methanol, and the solution was filtered over DOWEX 1X8, Cl^- form. After evaporation of the solvent the residue was crystallized from methanol/diethyl ether and dried at 90°C in vacuo: **17(X=Cl)** was obtained as silky-shining yellowish platelets of m.p. 257–258°C (dec.). – MS: *m/z* (%) = 387 (3, M^+, 1 Cl), 340 (43, 1 Cl), 281 (65), 260 (100), 167 (38). – ^{1}H NMR (360 MHz, [D$_4$]methanol): δ = 1.46–1.58 (m, 1H), 2.01 (s, 3H, CH_3CO-), 2.23–2.40 (m, 2H), 2.55–2.72 (m, 2H), 2.86–2.95 (m, 1H), 3.08–3.15 (m, 1H), 4.37–4.80 (m, 3H), 5.99–6.07 (m, 1H, $-CH=CH-CH_2-CH_2-$), 6.26 (d, J = 11.3 Hz, 1H, $-CH=CH-CH_2-CH_2-$), 7.47 (d, J = 7.7 Hz, 1H, ar-6-H), 7.54 (d, J = 7.9 Hz, 1H, ar-8-H), 7.74–7.79 (m, 1H, ar-7-H), 7.99–8.03 (m, 1H, pyr-17-H), 8.41 (d, $J \approx$ 8.0 Hz, 1H, pyr-16-H), 8.53 (br. s, 1H, pyr-20-H), 8.72 (d, $J \approx$ 5.0 Hz, 1H, pyr-18-H). – $C_{20}H_{22}ClN_3O_3$ (387.87): calcd. C 61.93, H 5.72, Cl 9.14, N 10.83; found C 61.82, H 5.93, Cl 9.41, N 11.07.

10-Nitro-4-aza[4]metacyclo[4](3,1)pyridiniophan-11-ene Chloride (**18,X=Cl**): A solution of 2.39 g (4.99 mmol) of **17(X=I)** in 9 ml of water and 5 ml of methanol was filtered through a short column of DOWEX 1X8 (Cl^- form) to convert the iodide into the corresponding chloride. The solvents were evaporated, and the residue was dissolved in 20 ml of concd. hydrochloric acid, 8 ml of water and 1 ml of methanol. This reaction mixture was heated at reflux under argon for 90 min. The solvents were again distilled off in vacuo to yield the crude deacylation product the solution of which in 50 ml of water was washed with 100 ml of diethyl ether, adjusted to pH 5–6 by addition of 2 N aqueous sodium hydroxide solution, saturated with sodium chloride and extracted four times with 100 ml each of 1-butanol. The combined 1-butanol extracts were washed with 100 ml of a concd. sodium chloride solution, filtrated and concentrated by evaporating the solvent in vacuo. The resulting solid product was extracted twice with 800 ml each of dichloromethane. The combined solutions were filtered and the filtrate was concentrated by distilling off dichloromethane until a volume of 40 ml was reached. After addition of 10 ml of diethyl ether the solution was kept at –18°C for 24 h for crystallization. The precipitate was then sucked off and washed with diethyl ether; yield: 1.51 g (88%) of **18(X=Cl)** as a brick-red crystal powder which was recrystallized from dichloromethane/diethyl ether: m.p. 240°C (dec.). – MS: *m/z* (%) = 345 (1, M^+), 310 (14), 292 (11), 282 (100), 274 (43), 144 (21), 93 (38) a.o. – ^{1}H NMR (360 MHz, [D$_4$]methanol): δ = 2.18–2.50 (m, 3H), 2.55–2.69 (m, 2H), 2.87–2.97 (m, 1H), 3.56–3.65 (m, 1H), 3.93–4.01 (m, 1H), 4.61–4.72 (m, 2H), 5.76–5.84 (m, 1H, 12-H), 6.45 (d, J = 7.1 Hz, 1H, ar-6-H), 6.82 (d, J = 11.2 Hz, 1H, 11-H), 7.01 (d, J = 8.5 Hz, 1H, ar-8-H), 7.33–7.38 (m, 1H, ar-7-H), 7.77–7.81 (m, 1H, pyr-17-H), 8.16 (d, J = 7.9 Hz, 1H, pyr-16-H), 8.32 (br. s, 1H, pyr-20-H), 8.86 (d, J = 5.9 Hz, 1H, pyr-18-H). – $C_{18}H_{20}ClN_3O_2$ (345.83): calcd. C 62.52, H 5.83, Cl 10.25, N 12.15; found C 62.50, H 5.99, Cl 10.53, N 12.00.

[4](3,1)Pyridinio[3](10,6)isoalloxazinophane Chloride (**4,X=Cl**) *and Perchlorate* (**4,X=ClO$_4$**): The solution of 185 mg (0.535 mmol) of **18(X=Cl)** in 6 ml of 1 N hydrochloric acid was added to a prehydrogenated suspension of 250 mg of palladium oxide in 6 ml of methanol, and the hydrogenation was performed at 20°C/760 Torr until the required 4 equiv. were taken up (51.5 ml; ca. 50 min). Then, by means of a reverse frit, the colorless solution was filtered under argon into a suspension of 200 mg (1.25 mmol) of alloxan hydrate in 5 ml of concd. hydrochloric acid; the catalyst was washed with a small amount of 2 N hydrochloric acid. The reaction mixture, changing immediately to a red color, was heated under nitrogen for 1 h to 70–75°C and then for 1 h to 90–95°C. The solvent was evaporated in vacuo at 70–75°C, and to the residue twice 5 ml each of absolute ethanol was added and the ethanol was distilled off from the solution. The remaining solid was dissolved in 30 ml of water, the solution was washed with 20 ml of 1-pentanol and twice with 15 ml each of 1-butanol, and the water was removed in a rotary evaporator. The product was dissolved in 5 ml of hot absolute ethanol and kept for 12 h at –18°C. After filtration the solvent was evaporated, and the resulting solid was dissolved in 4 ml of methanol and the solution chromatographed on a Sephadex LH20 column (h = 160 cm, d = 4 cm) from methanol controlled by TLC (silica gel; methanol/aqueous ethylammonium chloride solution/water; 10:1:0.5). With $R_f \approx$ 0.25, 24 ml of an intensely yellow solution with green fluorescence was isolated. After evaporation of the solvents the product was crystallized from ethanol to which a small amount of diethyl ether was added: thin yellow needles, dec. >251°C; 102 mg (yield 43%) of **4(X=Cl)**. – MS: *m/z* (%) = 425 (1), 389 (100), 387 (40), 347 (22), 317 (26), 120 (24), 106 (64), 93 (25). – ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 1.40–1.54 (m, 1H), 1.68–1.97 (m, 4H), 2.44–2.54 (m, 1H), 2.67–2.85 (m, 3H), 3.54–3.62 (m, 1H), 4.49–4.65 (m, 2H, 13-H), 4.69–4.78 (m, 1H, 11-H), 5.23–5.34 (m, 1H, 11-H), 7.56 ("d", $J \approx$ 7.2 Hz, 1H, 7-H), 7.72–7.76 (m, 1H, 16-H), 7.80 (br. s, 1H, 19-H), 7.88–7.92 (m, 1H, 8-H), 8.01 ("d", $J \approx$ 8.7 Hz, 1H, 9-H), 8.13 ("d", $J \approx$ 7.9 Hz, 1H, 17-H), 8.75 ("d", $J \approx$ 6.0 Hz, 1H, 15-H), 11.46 [s, 1H, H–N(3)]. – $C_{22}H_{22}ClN_5O_2 \cdot H_2O$ (441.92): calcd. C 59.79, H 5.47, Cl 8.02, N 15.85; found C 59.93, H 5.69, Cl 8.40, N 15.86.

Correct elemental analyses and an X-ray structure analysis of the **4**-system were performed when a sample of **4(X=Cl)**, dissolved in water, was filtered through a column of DOWEX 1X8 (perchlorate) and the product was crystallized from water to afford brown-yellow platelets of **4(X=ClO$_4$)**, dec. >280°C. For the results of the X-ray structure analysis of **4(X=ClO$_4$)** see above. – $C_{22}H_{22}ClN_5O_6$ (487.90): calcd. C 54.16, H 4.55, Cl 7.27, N 14.35; found C 53.97, H 4.80, Cl 7.35, N 14.54.

3-Acetylamino-2-nitrobenzyl Bromide (**20**): To a suspension of 11.7 g (55.7 mmol) of **19** and 36.0 g (109 mmol) of tetrabromomethane in 320 ml of dry dichloromethane 28.0 g (107 mmol) of triphenylphosphane was added in small portions, and the reaction mixture was kept with stirring for 16 h at 20°C. The solvent was evaporated under reduced pressure; the residue was dissolved in dichloromethane and filtrated through silica gel (h = 10 cm, d = 12 cm). By addition of *n*-hexane to the concentrated filtrate at –18°C 9.82 g (65%) of **20** was obtained as small yellow needles, m.p. 131–132°C (dec.). – MS: *m/z* (%) = 272 (26, M^+), 230 (100), 151 (33), 134 (27), 106 (20), 105 (19). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.22 (s, 3H, $-COCH_3$), 4.63 (s, 2H, $-CH_2Br$), 7.26 (d, J = 8.0 Hz, 1H, ar-4-H), 7.50 ("t", $J \approx$ 8.0 Hz, 1H, ar-5-H), 8.29 (d, J = 8.0 Hz, 1H, ar-6-H), 8.51 (br. s, 1H, NH). – $C_9H_9BrN_2O_3$ (273.09): calcd. C 39.58, H 3.32, Br 29.26, N 10.26; found C 39.64, H 3.10, Br 29.55, N 9.98.

3-Acetylamino-2-nitrobenzyl-triphenylphosphonium Bromide (**21**): A solution of 18.4 g (67.4 mmol) of **20** and 35.4 g (135 mmol) of triphenylphosphane in 500 ml of toluene was refluxed for 20 h. The precipitate formed was sucked off from the hot solution, washed three times with 60 ml each of hot toluene and twice with 50 ml each of diethyl ether and dried at 80°C under reduced pressure; yield: 35.3 g (98%) of **21** as a slightly yellowish

powder, dec. >254°C. – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.16 (s, 3H, $-COCH_3$), 5.25 (d, J = 14.2 Hz, 2H, $-CH_2PPh_3^+$), 6.99 ("t", $J \approx$ 7.9 Hz, 1H, ar-5-H), 7.15–7.18 (m, 1H, ar-H), 7.66–7.78 (m, 16H, ar-H), 10.34 (br. s, 1H, NH). – $C_{27}H_{24}BrN_2O_3P$ (535.38): calcd. C 60.57, H 4.52, Br 14.92, N 5.23, P 5.79; found C 60.50, H 4.36, Br 15.13, N 5.11, P 5.52.

3-(5-Methoxycarbonyl-3-pyridyl)propenal Dimethyl Acetal (**22**): A mixture of 33.5 g (155.1 mmol) of methyl 3-bromopyridine-5-carboxylate[18], 696 mg (3.1 mmol) of palladium(II) acetate, 3.78 g (12.4 mmol) of tri-*o*-tolylphosphane, 23.0 ml (190 mmol) of acrolein dimethyl acetal, 100 ml of triethylamine, and 50 ml of acetonitrile was heated under reflux for 16 h. After addition of 200 ml of diethyl ether the reaction mixture was filtrated through Florisil (h = 4 cm, d = 12 cm) and eluated with additional diethyl ether until no more product could be detected by TLC (R_f = 0.23; alumina, cyclohexane/ethyl acetate, 4:1). The solvents were evaporated, the residue was dissolved in a small amount of dichloromethane and chromatographed on alumina (h = 10, d = 12 cm): first, by elution with *n*-hexane/ethyl acetate (6:1), methyl 3-bromopyridine-5-carboxylate and tri-*o*-tolylphosphane were separated; then, with *n*-hexane/ethyl acetate (3:1), 13.5 g of a fraction containing crude **22** was obtained as a yellow oil (TLC: cyclohexane/ethyl acetate, 4:1, $R_f \approx$ 0.32) which was used for the following hydrogenation without further purification. – For the characterization of **22** chromatography was repeated for a second time furnishing 6.62 g (18%) of **22** as a colorless oil. – MS: m/z (%) = 237 (40, M^+), 206 (100, $[M - OMe]^+$). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 3.39 (s, 6H, $-OCH_3$), 3.97 (s, 3H, $-COOCH_3$), 5.01 [dd, J = 4.4 and 1.0 Hz, 1H, $-CH(OMe)_2$], 6.33 [dd, J = 16.1 and 4.4 Hz, 1H, $-CH{=}CH{-}CH(OMe)_2$], 6.78 [dd, J = 16.1 and 1.0 Hz, 1H, $-CH{=}CH{-}CH(OMe)_2$], 8.33 ("t", $J \approx$ 2.0 Hz, 1H, pyr-4-H), 8.77 [d, J = 2.0 Hz, 1H, pyr-2(or 6)-H], 9.09 [d, J = 2.0 Hz, 1H, pyr-6(or 2)-H]. – $C_{12}H_{15}NO_4$ (237.26): calcd. C 60.75, H 6.37, N 5.90; found C 60.74, H 6.51, N 5.91.

3-(5-Methoxycarbonyl-3-pyridyl)propanal Dimethyl Acetal (**23**): To a solution of 20.2 g (ca. 43 mmol) of crude **22** in 300 ml of methanol a suspension of 2.0 g of palladium catalyst (10% on charcoal) in 2 ml of ethyl acetate was added. After argon had been passed through the apparatus, hydrogenation was performed by bubbling hydrogen through the mixture for 6 h while heating at reflux. The catalyst was filtered off and washed with 250 ml of hot methanol in three portions. Evaporation of the solvent yielded 18.7 g of a mixture of **23** and several by-products; this crude product was used for the subsequent reaction. – For the characterization of **23** chromatography on alumina with *n*-hexane/ethyl acetate (6:1 to 3:1) was used ($R_f \approx$ 0.18; alumina, *n*-hexane/ethyl acetate, 4:1) followed by bulb-to-bulb distillation (110–130°C, ≈0.01 Torr). – MS: m/z (%) = 239 (20, M^+), 208 (38), 177 (25), 150 (32), 75 (100). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 1.91–1.98 [m, 2H, $-CH_2-CH_2-CH(OMe)_2$], 2.75 (t, J = 8.0 Hz, 2H, pyr-CH_2–), 3.36 (s, 6H, $-OCH_3$), 3.95 (s, 3H, $-COOCH_3$), 4.36 (t, J = 5.6 Hz, 1H, $-CH(OMe)_2$), 8.13 ("t", $J \approx$ 2.0 Hz, 1H, pyr-4-H), 8.62 (d, J = 2.0 Hz, 1H, pyr-2-H), 9.05 (d, J = 2.0 Hz, 1H, pyr-6-H). – $C_{12}H_{17}NO_4$ (239.27): calcd. C 60.24, H 7.16, N 5.85; found C 60.26, H 7.12, N 6.02.

3-(5-Methoxycarbonyl-3-pyridyl)propanal (**24**): A solution of 12.0 g of the crude product of the preceding reaction (containing ca. 25 mmol of **23**) in 75 ml of dichloromethane was stirred with 75 ml of 2 N hydrochloric acid for 16 h at 20°C. With ice cooling a saturated aqueous sodium carbonate solution was added to adjust the pH of the solution to 8.5–9.0. The aqueous layer was separated, saturated with sodium chloride and extracted four times with 50 ml each of dichloromethane. The combined extracts were dried with anhydrous magnesium sulfate, and the solvent was removed in vacuo to afford 7.58 g of a yellow oil containing **24** together with 3-(5-methoxycarbonylpyridyl-3)propyl methyl ether in variable amounts. Attempts to purify **24** by chromatography on silica gel and alumina with a variety of solvent combinations failed due to the decomposition of **24**. For the following Wittig reaction, therefore, the crude product was used. – Analytical data assigned to **24**: MS: m/z (%) = 193 (78, M^+), 164 (100) a.o. – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.86 (t, J = 7.3 Hz, 2H, $-CH_2-CH_2-$), 3.03 (t, J = 7.3 Hz, 2H, $-CH_2-CH_2-$), 3.95 (s, 3H, $-COOCH_3$), 8.10–8.14 (m, 1H, pyr-4-H), 8.59–8.65 (m, 1H, pyr-2-H), 9.04–9.07 (m, 1H, pyr-6-H), 9.83 (s, 1H, –CHO). – For an elemental analysis **24** was converted by standard methods into the corresponding 2,4-dinitrophenylhydrazone: orange solid, m.p. 152–153°C. – $C_{16}H_{15}N_5O_6$ (373.33): calcd. C 51.48, H 4.05, N 18.76; found C 51.52, H 4.13, N 18.56.

(Z)- and (E)-1-(3-Acetylamino-2-nitrophenyl)-4-(5-methoxycarbonyl-3-pyridyl)butene-1 [(*Z*)- and (*E*)-**25**]: A mixture of 16.3 g (281 mmol) of anhydrous potassium fluoride and 513 mg (1.42 mmol) of dibenzo-[18]crown-6 was stirred under argon at 120°C for 30 min. After addition of 100 ml of dry acetonitrile and stirring for further 20 min, 8.26 g (15.4 mmol) of **21** was added (deep blue color). A solution of 7.58 g of crude **43** (ca. 14 mmol according to ^{1}H NMR) in 100 ml of dry acetonitrile was added dropwise over 30 min, and the reaction mixture was kept at 80°C with stirring for 6 h. Then under the same conditions further 400 mg (0.75 mmol) of **21** and after 2 h again 400 mg (0.75 mmol) of **21** were added. After stirring at 80°C for additional 4 h the precipitate (inorganic salts) was filtered off and washed four times with 40 ml each of trichloromethane. From the combined organic solutions the solvents were evaporated yielding a brown oil which was dissolved in a small amount of dichloromethane and chromatographed on silica gel (h = 9 cm, d = 12 cm); unpolar by-products were eluated with ethyl acetate/cyclohexane (1:1), then with ethyl acetate/cyclohexane (increasing from 2:1 to 4:1) the fraction containing the product was eluated. Flash chromatography (silica gel; h = 20 cm, d = 7 cm; trichloromethane/ethyl acetate, 5:1) allowed the separation of the last traces of triphenylphosphane oxide and a partial separation of the *Z* and *E* isomers of **25**; overall yield of **25**: 4.48 g (87%), *Z*:*E* ratio ca. 3:2. By crystallization from diethyl ether at −18°C a yellow precipitate of **25** with an enrichment of the *E* isomer (*E*:*Z* ratio 82:18) was obtained. Further concentration of the solution yielded a colourless precipitate enriched with the *Z* isomer (*Z*:*E* ≈ 4:1; m.p. 98–99°C). – MS (*Z*,*E* mixture): m/z (%) = 369 (70, M^+), 354 (70), 326 (30), 323 (30), 187 (20), 177 (65), 165 (100), 164 (50), 151 (60), 135 (25), 131 (60). – $C_{19}H_{19}N_3O_5$ (369.38): calcd. C 61.78, H 5.18, N 11.38; found C 61.82, H 5.24, N 11.26.

^{1}H NMR of **25**, *Z* isomer (500 MHz, $CDCl_3$): δ = 2.21 (s, 3H, $-COCH_3$), 2.39–2.43 (m, 2H, 3-H), 2.75 (t, J = 7.4 Hz, 2H, 4-H), 3.95 (s, 3H, $-COOCH_3$), 5.80 (dt, J = 11.4 and 7.4 Hz, 1H, 2-H), 6.51 (d, J = 11.4 Hz, 1H, 1-H), 6.87 (d, J = 8.0 Hz, 1H, ar-6-H), 7.44 ("t", J = 8.0 Hz, 1H, ar-5-H), 8.02 ("t", $J \approx$ 2.0 Hz, 1H, pyr-4-H), 8.22 (d, J = 8.0 Hz, 1H, ar-4-H), 8.49 (br. s, 1H, NH), 8.54 (d, J = 2.0 Hz, 1H, pyr-2-H), 9.05 (d, J = 2.0 Hz, 1H, pyr-6-H).

^{1}H NMR of **25**, *E* isomer (360 MHz, $CDCl_3$): δ = 2.19 (s, 3H, $-COCH_3$), 2.56–2.62 (m, 2H, 3-H), 2.88 (t, J = 7.6 Hz, 2H, 4-H), 3.96 (s, 3H, $-COOCH_3$), 6.19 (dt, J = 15.6 and 6.8 Hz, 1H, 2-H), 6.44 (d, J = 15.6 Hz, 1H, 1-H), 7.25 (d, J = 8.0 Hz, 1H, ar-6-H), 7.44 ("t", $J \approx$ 8.0 Hz, 1H, ar-5-H), 8.10–8.18 (m, 2H, pyr-4-H and ar-4-H), 8.27 (br. s, 1H, NH), 8.63 (d, J = 2.0 Hz, 1H, pyr-2-H), 9.08 (d, J = 2.0 Hz, 1H, pyr-6-H).

(Z) and (E)-1-(Acetylamino-2-nitrophenyl)-4-(5-carbamoyl-3-pyridyl)-butene-1 [(*Z*)- and (*E*)-**26**]: To a suspension of 1.62 g (4.39 mmol) of **25** (*Z*:*E* ratio ca. 4:1 according to ^{1}H-NMR analysis) in 35 ml of methanol, 135 ml of an aqueous ammonia solution (30%) was added at −10°C, and for 6 h a stream of gaseous ammonia was passed through the mixture at −10°C. The reaction mixture then was kept for 16 h at room temp. The colorless precipitate formed was sucked off, washed with water and dried at 80°C/1 mbar to yield 1.0 g of the nearly pure (*Z*)-**26** (yield ca. 80% relative to the *Z* content of the starting material), colorless powder, m.p. 175–177°C. In an analogous way, starting from **25** enriched with the *E* isomer, we obtained pure (*E*)-**26** as a yellow powder of m.p. 155–157°C.

(*Z*)-**26**: MS: m/z (%) = 354 (20, M^+), 339 (45), 308 (22), 177 (52), 150 (100), 149 (39), 136 (43), 135 (28), 131 (40), 130 (22). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ = 2.00 (s, 3H, $-COCH_3$), 2.38–2.42 (m, 2H, 3-H), 2.75 (t, J = 7.6 Hz, 2H, 4-H), 5.87 (dt, J = 11.5 and 7.3 Hz, 1H, 2-H), 6.37 (d, J = 11.5 Hz, 1H, 1-H), 7.07 [d, J = 7.8 Hz, 1H, ar-4(or 6)-H], 7.44 [dd, J = 7.8 and 1.3 Hz, 1H, ar-6(or 4)-H], 7.49 (br. s, 1H, NH), 7.51 ("t", $J \approx$ 7.8 Hz, 1H, ar-5-H), 8.01 ("t", $J \approx$ 2.1 Hz, 1H, pyr-4-H), 8.06 (br. s, 1H, NH), 8.51 (d, J = 2.1 Hz, 1H, pyr-2-H), 8.85 (d, J = 2.1 Hz, 1H, pyr-6-H), 9.92 (br. s, 1H, –NHAc). – $C_{18}H_{18}N_4O_4$ (354.37): calcd. C 61.01, H 5.12, N 15.81; found C 61.30, H 5.01, N 15.83.

(*E*)-**26**: MS: m/z (%) = 354 (25, M^+), 339 (28), 177 (60), 150 (100), 149 (48), 136 (52), 135 (55), 131 (56), 130 (30). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ = 1.99 (s, 3H, $-COCH_3$), 2.53–2.59 (m, 2H, 3-H), 2.83 (t, J = 7.5 Hz, 2H, 4-H), 6.27 (d, J = 15.6 Hz, 1H, 1-H), 6.43 (dt, J = 15.6 and 6.7 Hz, 1H, 2-H), 7.39–7.41 (m, 1H, ar-H), 7.49–7.53 (m, 3H, ar-H, NH), 8.06 (br. s, 1H, NH), 8.09 ("t", $J \approx$ 2.0 Hz, 1H, pyr-4-H), 8.58 (d, J = 2.0 Hz, 1H, pyr-2-H), 8.85 (d, $J \approx$ 2.0 Hz, 1H, pyr-4-H), 9.84 (br. s, 1H, –NHAc). – $C_{18}H_{18}N_4O_4$ (354.37): calcd. C 61.01, H 5.12, N 15.81; found C 60.80, H 5.32, N 15.83.

(Z)- and (E)-1-{3-[Acetyl(3-chloropropyl)amino]-2-nitrophenyl}-4-(5-carbamoyl-3-pyridyl)butene-1 [(*Z*)- and (*E*)-**27**]: To a suspension of 250 mg (4.46 mmol) of potassium hydroxide powder in 12 ml of dry DMSO, stirred under argon for 5 min, 1.05 g (2.96 mmol) of **26** (*Z*:*E* ratio ca. 85:14, according to ^{1}H-NMR analysis) was added, and the solution was cooled until DMSO began to solidify (ca. 10°C). After 2.00 ml (20.3 mmol) of 1-bromo-3-chloropropane had been added within 1 min, the reaction mixture was stirred for 3 h at 10–12°C. The solution was poured into a mixture of 20 g of crushed ice, 20 ml of a saturated aqueous ammonium chloride solution and 40 ml of dichloromethane. The aqueous layer was separated and extracted three times with 30 ml each of dichloromethane; the combined organic phases were washed twice with 10 ml each of water and with 20 ml each of brine, then dried with anhydrous magnesium sulfate. By flash chromatography (silica gel; h = 12 cm, d = 7 cm; ethyl acetate/ethanol, 9:1) the fraction with $R_f \approx$ 0.20 yielded 1.14 g (89%) of an isomer mixture of **27** (*Z*/*E* ratio ca. 4:1, determined by ^{1}H-NMR analysis) as a yellow oil which was directly used for the cyclization to **28**.

(11Z)-4-Acetyl-10-nitro-4-aza[4]metacyclo[4](5,1)pyridiniophan-11-ene-17-carboxamide Iodide (**28,X=I**): A solution of 1.13 g (2.62 mmol) of the aforementioned *Z/E* isomer mixture of **27** in 170 ml of dry DMF was added during 16 h by means of a high-dilution apparatus[21] under argon to a refluxing solution of 8.50 g (5.12 mmol) of potassium iodide in 150 ml of dry DMF and 75 ml of dry acetone. After stirring for additional 6 h the solution was concentrated under reduced pressure to a volume of ca. 5 ml; this concentrate was extracted with 600 ml of boiling trichloromethane, the undissolved residue was washed four times with 100 ml each of hot trichloromethane and treated with 20 ml of boiling methanol. After further addition of 300 ml of hot trichloromethane the insoluble precipitate was filtered off and the combined organic extracts were concentrated. The solid residue was dissolved in 250 ml of water, and the solution was extracted four times with 50 ml each of ethyl acetate. The residue, obtained from the aqueous solution by the evaporation of water, was dissolved in 10 ml methanol/water (1:1) and chromatographed on Sephadex LH 20 (h = 140 cm, d = 4 cm) with methanol. Subsequent recrystallization from water and drying at 80°C in vacuo yielded 666 mg (61%, referred to of (*Z*)-**27**): yellow solid, m.p. 265–267°C. – MS (LSI-MS): *m/z* (%) = 395 (100, M–I). – ^{1}H NMR (500 MHz, D_2O): δ = 1.44–1.50 (m, 1H, 2-H), 2.08 (s, 3H, $-COCH_3$), 2.35–2.45 (m, 2H, 2,3-H), 2.62–2.66 (m, 2H, 13-H), 2.92–2.98 (m, 1H, 14-H), 3.12–3.17 (m, 1H, 14-H), 4.44–4.75 (m, 3H, 1,3-H), 6.04–6.10 (m, 1H, 12-H), 6.33 (d, J = 11.4 Hz, 1H, 11-H), 7.54 [d, J = 8.0 Hz, 1H, ar-6(or 8)-H], 7.55 [d, J = 8.0 Hz, 1H, ar-8(or 6)-H)], 7.79 ("t", $J \approx$ 8.0 Hz, 1H, ar-7-H), 8.63 (s, 1H, pyr-16-H), 8.78 (s, 1H, pyr-20-H), 9.08 (s, 1H, pyr-18-H). – $C_{21}H_{23}IN_4O_4$ (522.34): calcd. C 48.29, H 4.44, N 10.73; found C 48.48, H 4.44, N 10.62.

By anion exchange (Sephadex DEAE A-25, Cl^- form, and Dowex 1 × 8, Br^- form, respectively) the corresponding chloride **29** and bromide **30** were obtained; an X-ray structure analysis of the bromide **30** was reported above.

Methyl (11Z)-10-Nitro-4-Aza[4]metacyclo[4](5,1)pyridiniophan-11-ene-17-carboxylate Bromide (**32**): To a solution of 570 mg (1.32 mmol) of **29** in 40 ml of dry methanol 2.0 ml of acetyl chloride was added dropwise with ice cooling, and the reaction mixture was heated at reflux under argon for 18 h. The solvent was evaporated, and to the residue 2 ml of 2 N triethylammonium acetate buffer pH 7 was added. After adjustiment of the pH of the mixture to 8 with 2 N aqueous ammonia, ion exchange chromatography (Sephadex SP C-25, triethylammonium form) with water as the eluant yielded ca. 100 mg of a mixture of the zwitterionic **31** (see below) and its *N*-acetyl derivative. As the main product of this reaction we obtained after elution with 0.2 M triethylammonium acetate solution (pH 7) and chromatography from water on Sephadex DEAE A-25, (bromide form), 209 mg (35%) of **32** as orange crystals, m.p. 215°C (from water). – MS (LSI-MS): *m/z* (%) = 368 (100). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.98–2.08 (m, 1H, 13-H), 2.11–2.19 (m, 1H, 2-H), 2.35–2.43 (m, 1H, 13-H), 2.44–2.50 (m, 1H, 2-H), 2.72–2.83 (m, 2H, 14-H), 3.57–3.65 (m, 1H, 3-H), 3.86–3.93 (m, 1H, 3-H), 4.02 (s, 3H, $-COOCH_3$), 4.58–4.64 (m, 1H, 1-H), 4.72–4.80 (m, 1H, 1-H), 5.74–5.81 (m, 1H, 12-H), 6.39 (d, J = 6.9 Hz, 1H, ar-8-H), 6.75 (d, J = 11.3 Hz, 1H, 11-H), 7.08 (d, J = 8.5 Hz, 1H, ar-6-H), 7.31 ("t", $J \approx$ 6.6 Hz, 1H, NH), 7.39 ("t", $J \approx$ 7.8 Hz, 1H, ar-7-H), 8.60 (s, 1H, pyr-20-H), 8.80 (s, 1H, pyr-16-H), 9.54 (s, 1H, pyr-18-H). – $C_{20}H_{22}BrN_3O_4$ (448.32): calcd. C 53.58, H 4.95, N 9.37; found C 53.35, H 4.85, N 9.47.

(11Z)-17-Carbamoyl-10-nitro-4-aza[4]metacyclo[4](5,1)pyridiniophan-11-ene Bromide (**33**): With exclusion of moisture and cooling by ice a stream of ammonia was passed through 5 ml of dry methanol. Then 42.0 mg (93.8 μmol) of **32** was added and the mixture was stirred by bubbling continuously ammonia through it and cooling with ice for 4 h. After standing at room temp. for 16 h the precipitate formed was sucked off, washed with 10 ml of diethyl ether, and the filtrate was concentrated by evaporation to a volume of 2 ml. Then 10 ml of diethyl ether was added; after 2 h at –5°C the precipitate was filtered off, combined with the previously separated raw product, dissolved in water and the solution was filtrated through Sephadex DEAE A-25 (bromide form). The fraction containing the product was freeze-dried and then recrystallized from water to afford 28.4 mg (70%) of **33**, m.p. 290°C (dec.); $R_f \approx$ 0.31 (silica gel, methanol/acetic acid, 100:1). – MS (LSI-MS): *m/z* (%) = 353 (100, M–Br). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 2.09–2.17 (m, 1H, 13-H), 2.19–2.27 (m, 1H, 2-H), 2.36–2.45 (m, 1H, 13-H), 2.46–2.57 (m, 1H, 2-H), 2.57–2.65 (m, 1H, 14-H), 2.80–2.85 (m, 1H, 14-H), 3.50–3.58 (m, 1H, 3-H), 3.85–3.93 (m, 1H, 3-H), 4.53–4.59 (m, 1H, 1-H), 4.64–4.70 (m, 1H, 1-H), 5.73–5.79 (m, 1H, 12-H), 6.40 (d, J = 7.0 Hz, 1H, ar-8-H), 6.72 (d, J = 11.2 Hz, 1H, 11-H), 7.06 (d, J = 8.5 Hz, 1H, ar-6-H), 7.21 ("t", $J \approx$ 6.6 Hz, 1H, NH), 7.38 ("t", $J \approx$ 7.8 Hz, 1H, ar-7-H), 8.11 (br. s, 1H, $-CONH_2$), 8.34 (br. s, 1H, $-CONH_2$), 8.57 (s, 1H, pyr-16-H), 8.61 (s, 1H, pyr-20-H), 9.32 (s, 1H, pyr-18-H). – $C_{19}H_{21}BrN_4O_3$ (433.30): calcd. C 52.67, H 4.88, N 12.93; found C 52.57, H 4.93, N 12.77.

(11Z)-10-Nitro-4-aza[4]metacyclo[4](5,1)pyridiniophan-11-ene-17-carboxylate (**31**): An aqueous solution of 211 mg (404 μmol) of **28** was filtered through Dowex 1 × 18 (chloride form; h = 10 cm, d = 3 cm) to obtain the aqueous solution of the chloride **29**. After evaporation of the water under reduced pressure the colorless solid obtained was dissolved in 19 ml of 10% aqueous hydrochloric acid and the solution heated at reflux under argon for 2.5 h. The solvent was distilled off in vacuo, and the residue was treated with 2 ml of water to yield an orange precipitate which was dissolved by dropwise addition of 2 N aqueous ammonia, and the solution was filtered with water over Sephadex DEAE A-25 (OH^- form; h = 7 cm, d = 5 cm). The fraction containing the product was freeze-dried and crystallized from methanol to furnish 101 mg (71%) of **31** as an orange solid, m.p. 245°C (dec.). – MS (LSI-MS): *m/z* (%) = 354 (100, MH^+), 353 (7, M^+). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 2.04–2.13 (m, 1H, 13-H), 2.18–2.25 (m, 1H, 2-H), 2.30–2.42 (m, 2H, 2,13-H), 2.46–2.56 (m, 1H, 14-H), 2.78–2.85 (m, 1H, 14-H), 3.38–3.46 (m, 1H, 3-H), 3.70–3.77 (m, 1H, 3-H), 4.47–4.54 (m, 1H, 1-H), 4.65–4.73 (m, 1H, 1-H), 5.78–5.84 (m, 1H, 12-H), 6.41 (d, J = 7.0 Hz, 1H, ar-8-H), 6.63 (d, J = 11.2 Hz, 1H, 11-H), 6.95 ("t", $J \approx$ 6.7 Hz, 1H, NH), 7.00 (d, J = 8.5 Hz, 1H, ar-6-H), 7.31 ("t", $J \approx$ 7.8 Hz, 1H, ar-7-H), 8.17 (s, 1H, pyr-20-H), 8.39 (s, 1H, pyr-16-H), 9.10 (s, 1H, pyr-18-H). – $C_{19}H_{19}N_3O_4 \cdot CH_3OH$ (385.42): calcd. C 62.33, H 6.01, N 10.90; found C 62.13, H 5.71, N 10.88.

[4](5,1)Pyridinio[3](10,6)isoalloxazinophane-16-carboxylate (**34**): Through a mixture of 4.5 ml of methanol and 0.5 ml of 1 N hydrochloric acid in a 50 ml flask hydrogen was passed in the presence of 61 mg of palladium catalyst (10% on charcoal, oxidic form). Then a solution of 108 mg (306 μmol) of **31** in 5 ml of the same solvent mixture was added and hydrogenated at 20°C/1 bar up to a hydrogen uptake of 36.7 ml (≈ 5 equiv.; ca. 60 min). The solution was filtrated under argon into a suspension of 247 mg (1.54 mmol) of alloxan hydrate in 1 ml of concd. (32%) hydrochloric acid, the catalyst was rinsed with additional 4 ml of concd. hydrochloric acid, and the reaction mixture was stirred for 2 h at 40°C and then for 30 min at 60°C. After the mixture had been kept for 16 h at room temp. the solvent was distilled off in vacuo, the residue was treated with 2 ml of 2 N triethylammonium acetate buffer pH 7, and the pH of the mixture was adjusted to 7 to 8 by addition of triethylamine. Then 5 ml of water was added, the solution was extracted four times with 10 ml each of trichloromethane, and from the aqueous phase the water was evaporated. The residue was dissolved in 4 ml of methanol and the solution chromatographed on Sephadex LH 20 (h = 140 cm, d = 4 cm) with methanol. The solvent was evaporated in vacuo, the residue dissolved in 1 ml water, and the resulting solution was overlayered by 20 ml of acetone. The precipitate (840 mg) formed after the mixture had been kept at 5°C for 16 h was again chromatographed with water over Biogel P2 to yield after freeze-drying 37.2 mg of a light yellow solid. By preparative HPLC (MN Nucleosil NMe2, 10 μm, 20 × 250 mm, 15.0 ml/min, acetonitrile/water from 9:1 to 8:2: t_R = 10.1 min) a uniform product was obtained which was crystallized from acetone/water; yield of **34**: 13.8 mg (11%) dec. >230°C. – MS (LSI-MS): *m/z* = 433 (44, MH_2^+), 432 (100, MH^+), 431 (10, M^+). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.59–1.76 (m, 2H, 21,22-H), 1.77–1.85 (m, 1H, 21-H), 1.86–2.04 (m, 2H, 20,22-H), 2.41–2.55 (m, 1H, 20-H), 2.67–2.93 (m, 3H, 12,23-H), 3.55–3.60 (m, 1H, 23-H), 4.48–4.60 (m, 2H, 13-H), 4.65–4.74 (m, 1H, 11-H), 5.23–5.32 (m, 1H, 11-H), 7.47 (d, J = 7.3 Hz, 1H, flav-7-H), 7.56 (s, 1H, pyr-19-H), 7.76 ("t", $J \approx$ 7.9 Hz, 1H, flav-8-H), 7.91 (d, J = 8.7 Hz, 1H, flav-9-H), 8.21 (s, 1H, pyr-17-H), 8.81 (s, 1H, pyr-15-H), 11.35 (s, 1H, NH). – For an X-ray structure analysis **34** see above. – $C_{23}H_{21}N_5O_4$: calcd. 431.1593 (M^+), found 431.1564 (HR-MS); calcd. 432.1672 (MH^+); found 432.1650 (HR-MS).

16-Methoxycarbonyl[4](5,1)pyridinio[3](10,6)isoalloxazinophane Chloride (**2,X=Cl**): A solution of 10.3 mg (23.9 μmol) of **34** and 0.25 ml of acetyl chloride in 5 ml of dry methanol was heated at reflux for 3 h under argon and with exclusion of light. After cooling of the solution to 5°C, 9.1 mg (79%) of nearly pure **2(X=Cl)** was precipitated, filtered off and washed with diethyl ether. Recrystallization from methanol yielded 4.0 mg (35%) of pure **2(X=Cl)** as fine yellow needles, m.p. 252°C. – MS (LSI-MS): *m/z* (%) = 448 (14), 447 (50), 446 (100). – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 1.08–1.18 (m, 1H, 22-H), 1.68–1.80 (m, 3H, 21,22-H), 2.05–2.12 (m, 1H, 20-H), 2.50–2.60 (m, 1H, 20-H), 2.65–2.72 (m, 1H, 23-H), 2.77–2.85 (m, 2H, 12-H), 3.53–3.62 (m, 1H, 23-H), 4.00 (s, 3H, $-COOCH_3$), 4.52–4.60 (m, 1H, 13-H), 4.67–4.74 (m, 1H, 11-H), 4.80–4.85 (m, 1H, 13-H), 5.33–5.40 (m, 1H, 11-H), 7.53 (d, J = 7.1 Hz, 1H, 7-H), 7.90 ("t", $J \approx$ 7.9 Hz, 1H, 8-H), 7.96 (d, J = 8.5 Hz, 1H, 9-H), 8.27 (s, 1H, 19-H), 8.53 (s, 1H, 17-H), 9.30 (s, 1H, 15-H), 11.48 (s, 1H, NH). – $C_{24}H_{24}ClN_5O_4$: calcd. 446.1828 (M^+); found 446.1840 (HR-MS), calcd. 447.1906 (MH^+); found 447.1900 (HR-MS).

N-Acetyl-3-methyl-2-nitroaniline: A mixture of 35.0 g (230 mmol) of 3-methyl-2-nitroaniline[26], together with 200 ml of acetic acid, 27 ml (264 mmol) of acetic anhydride, and 4 ml of concd. sulfuric acid was heated to 90°C for 40 min. The precipitate formed by pouring the cooled reaction mixture into 1500 ml of ice water was sucked off, and the filtrate was twice

extracted with 200 ml each of trichloromethane. The combined organic extracts were washed with 75 ml of a saturated aqueous hydrogen carbonate solution and twice with 100 ml each of water. After evaporation of the solvent the residue was combined with the precipitate mentioned above and crystallized from cyclohexane to afford 38.0 g (85%) of small colorless needles, m.p. 123.5–124°C. – $C_9H_{10}N_2O_3$ (194.19): calcd. C 55.67, H 5.19, N 14.43; found C 55.49, H 5.29, N 14.34.

3-Methylamino-2-nitrotoluene (**35**): To a stirred suspension of 7.9 g (120 mmol) of potassium hydroxide powder (85% KOH) and 9.7 g (50 mmol) of *N*-acetyl-3-methyl-2-nitroaniline in 30 ml of dry DMSO under argon and with ice cooling, 8.52 g (60 mmol) of iodomethane was added. After stirring for 2 h, 60 ml of water was added, and the mixture was heated to 100°C for 30 min, then poured onto 100 g of crushed ice. The solid formed was sucked off and the filtrate was extracted four times with 25 ml each of dichloromethane. The combined organic phases were dried with anhydrous magnesium sulfate, the solvent was evaporated, the residue was combined with the precipitate mentioned above, dissolved in dichloromethane and the solution was filtrated through silica gel. The product obtained by evaporation of the solvent (8.3 g) was suspended in a solution of 100 g of potassium hydroxide in 70 ml of water and 70 ml of ethanol and the suspension was heated under argon to 80–90°C for 40 min. The mixture was poured on 800 g of crushed ice, and the precipitate formed was sucked off after 15 min and dried. Filtration from a solution in dichloromethane and recrystallization from water/methanol (1:1) yielded 7.51 g (90%) of **35** as orange-yellow needles, m.p. 69.0–69.5°C. – MS: *m/z* (%) = 166 (100, M^+), 149 (30). – ^{1}H NMR (360 MHz, $CDCl_3$): δ = 2.48 (s, 3H, 3-CH_3), 2.94 (d, *J* = 5.1 Hz, 3H, N-CH_3), 6.52 (d, *J* = 7.4 Hz, 1H, ar-4-H), 6.59 (br. m, 1H, NH), 6.65 (d, *J* = 8.5 Hz, 1H, ar-6-H), 7.24 ("t", *J* ≈ 8.0 Hz, 1H, ar-5-H). – $C_8H_{10}N_2O_2$ (166.18): calcd. C 57.82, H 6.07, N 16.86; found C 57.66, H 6.07, N 16.79.

6,10-Dimethylisoalloxazine (**36**): A solution of 2.00 g (12.0 mmol) of **35** and 3.71 g (60 mmol) of boric acid in 100 ml of acetic acid was hydrogenated in the presence of 200 mg of palladium catalyst (10% on charcoal) at room temp./1 bar up to the end of hydrogen consumption. The solution was filtrated under nitrogen into a suspension of 6.42 g (30.0 mmol) of alloxan tetrahydrate and 7.42 g (120 mmol) of boric acid in 100 ml of acetic acid, and the mixture was stirred at 60°C under nitrogen in the dark for 19 h. The solvent was evaporated, the boric acid was removed by threefold distillation of the residue with 10 ml each of methanol and the solid, adsorbed on 2 g of Florisil, was chromatographed on silica gel (*h* = 80 cm, *d* = 5 cm; trichloromethane/methanol, 19:1; R_f = 0.30, green fluorescence). The product was reprecipitated from acetic acid/diethyl ether and dried at 150°C/10^{-3} Torr; yield: 1.08 g (37%) of **36**, yellow needles, m.p. >340°C. – MS: *m/z* (%) = 242 (100, M^+), 199 (52), 170 (21). – ^{1}H NMR (360 MHz, [D_6]DMSO): δ = 2.72 (s, 3H, 6-CH_3), 3.97 (s, 3H, 10-CH_3), 7.53 (d, *J* = 7.4 Hz, 1H, flav-7-H), 7.75 (d, *J* = 8.4 Hz, 1H, flav-9-H), 7.81–7.87 (m, 1H, flav-8-H), 11.33 (br. s, 1H, NH). – $C_{12}H_{10}N_4O_3$ (242.24): calcd. C 59.50, H 4.16, N 23.13; found C 59.71, H 3.96, N 23.20.

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