Pyridazine-Fused Carbazoles: Synthesis, Reactivity, and Antitumor Activity

Norbert Haider

Institute of Pharmaceutical Chemistry, University of Vienna Althanstraße 14, A-1090 Vienna, Austria

Dedicated to the memory of Professor Raymond N. Castle

J. Heterocyclic Chem., **39**, 511 (2002).

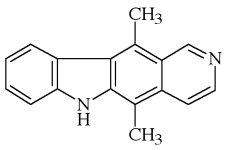
Introduction.

The alkaloid *ellipticine* (5,11-dimethyl-6*H*-pyrido-[4,3-*b*]carbazole) was isolated more than 40 years ago from the tropical plant, *ochrosia elliptica* [1]. The discovery of the antineoplastic activity of this tetracyclic compound and that of related natural products such as the isomer, *olivacine* (1,5-dimethyl-6*H*-pyrido[4,3-*b*]carbazole), has stimulated considerable research efforts in this field of condensed systems [2,3], leading to the development of new derivatives and analogs with an enhanced pharmacological profile. Examples are the drug molecules *elliptinium* [4], *datelliptium* [5], *retelliptine* [6], and *pazelliptine* [7], whose structures are given in Scheme 1.

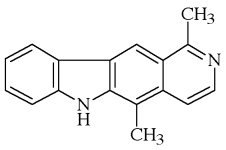
With respect to the mechanism of the observed antineoplastic activity of *ellipticine* and related compounds, several theories have emerged, starting with simple DNA intercalation models in the 1970's [8]. Later on, various possibilities for bio-activation pathways were discussed, for instance the oxidation of ring A into a quinone imine which then might react readily with nucleophilic centers in the DNA molecule [9]. Other authors proposed a metabolic pathway which involves hydroxylation of the methyl group in position 5, followed by esterification with phosphate which would generate a reactive alkylating agent, again with DNA structures as the target [10]. Today, it is widely accepted that the main mode of action of *ellipticine* is based on an interaction with the enzyme topoisomerase II [11], in terms of a stabilization of the "cleavable complex" which is formed between DNA and topoisomerase II during a particular stage of the cell cycle. Quite recently, an additional mechanism was proposed for certain *ellipticine* derivatives [12], which is entirely different and appears to be based on a regeneration of the functionality of the p53 protein which gets lost in many tumor tissues and which plays an important role in controlling the DNA integrity of a cell.

The structural modifications of the parent compound (see Scheme 1) are characterized, for instance, by introduction of oxygen functionalities in position 9, by quaternization of the pyridine nitrogen, or by attachment of a basic side chain onto C-1. Moreover, the pyrido-[3',4':4,5]pyrrolo[2,3-*g*]isoquinoline, *pazelliptine*, represents a 9-aza-*ellipticine* structure, with a nitrogen atom incorporated into ring A. Several 1,4-dialkoxy-pyridazino[4,5-*b*]carbazoles, representing 3-aza analogs of the pyrido[4,3-*b*]carbazole system, had been prepared in 1989 by Landelle *et al.*, but these compounds were found to be almost inactive in an antitumor screening [13]. Nevertheless, we started a reinvestigation of this ring system some years ago, and so far a series of new representatives of this type were synthesized, some of them exhibiting significant biological activity in preliminary screenings.

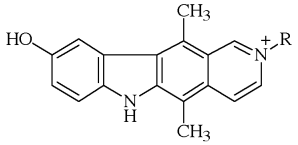
Scheme 1



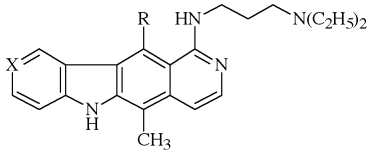
Ellipticine



Olivacine



Elliptinium R = CH_3
Datelliptium R = $CH_2CH_2N(C_2H_5)_2$



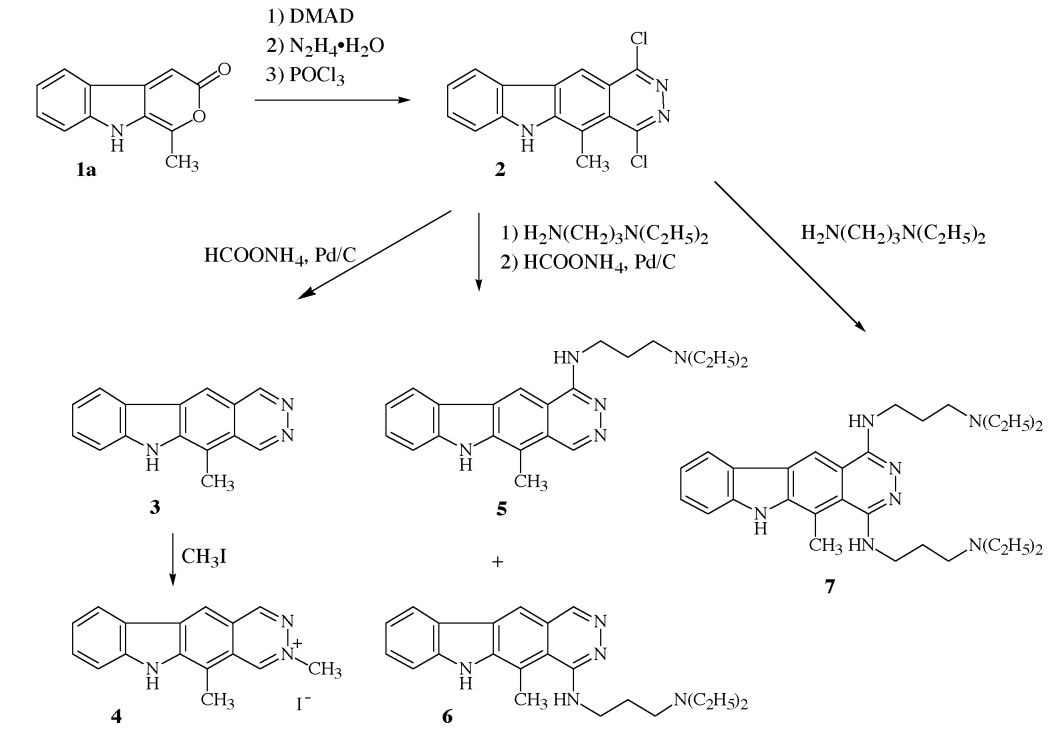
Retelliptine R = CH_3 X = $COCH_3$
Pazelliptine R = H X = N

Synthesis of 3-Aza-*Ellipticine* Analogs.

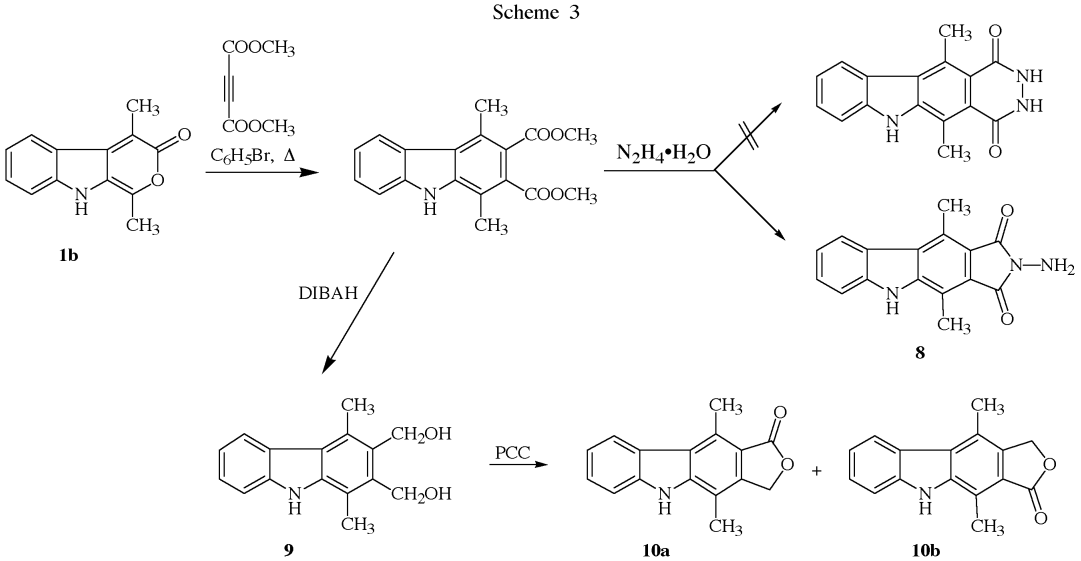
Utilizing the known 1,4-dichloropyridazino[4,5-*b*]-carbazole **2** [13] as the key intermediate, the amino-pyridazines **5**, **6**, and **7** were obtained as outlined in Scheme 2. Reductive dehalogenation of **2** gave the 3-aza-11-desmethyl-*ellipticine* **3**, which was converted into the quaternary salt **4** by treatment with methyl iodide. Among this group, the 1-substituted compound **5** showed the highest *in vitro* antineoplastic potency in the standard NCI screening panel of 60 different tumor cell lines. Its activity was only exceeded by that of a 9-methoxy congener which was prepared *via* an analogous pathway [14] and which in the meantime successfully passed the *in vivo* hollow-fiber assay.

Whereas 1-methyl-2,3-carbazoledicarboxylic acid esters smoothly afford the corresponding carbazole-fused pyridazinedione on treatment with hydrazine, the

Scheme 2



Scheme 3



1,4-dimethyl-2,3-carbazolediester [15] under the same conditions gives a five-membered cyclization product, *i.e*,. the *N*-aminoimide **8** (Scheme 3) [16]. Thus, the sequence used for the preparation of **3** could not be adopted for the synthesis of 3-aza-*ellipticine* (5,11-dimethylpyridazino-[4,5-*b*]carbazole). Therefore, several alternative synthetic pathways to this target structure were investigated.

First, we attempted to prepare the corresponding dialdehyde (which might cyclize in the expected way with hydrazine) by reduction of the diester with diisobutyl aluminium hydride, but this approach failed (Scheme 3). Under the conditions required for complete conversion of the ester, the corresponding dialcohol (**9**) was obtained. Not surprisingly, oxidation of **9** did not afford the dialdehyde, but gave a mixture of two isomeric lactones (**10a,b**).

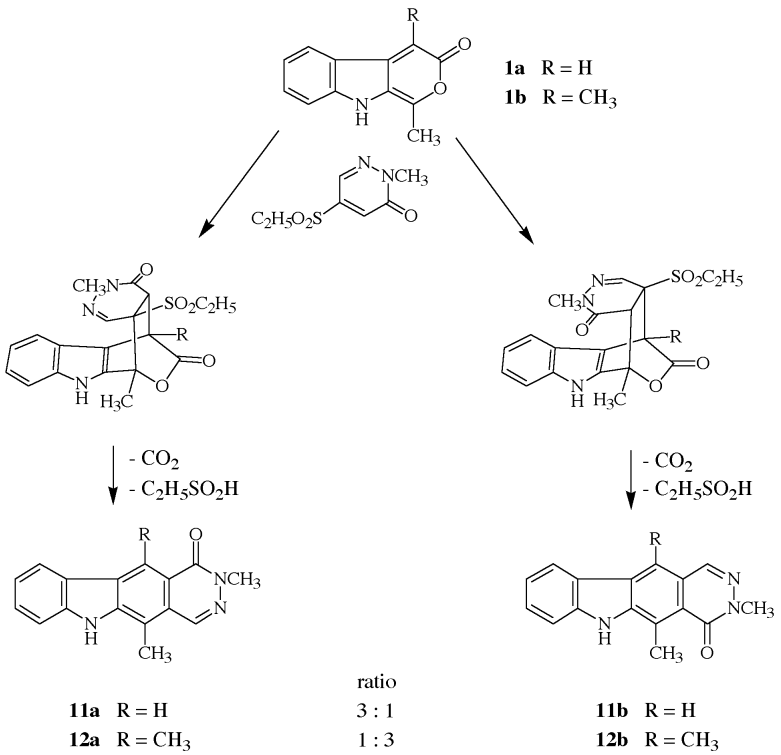
Next, we investigated the construction of the tetracyclic system by a cycloaddition strategy, employing a reactant with a preformed pyridazine unit [17]. 5-Ethanesulfonyl-2-methylpyridazin-3(2*H*)-one [18], which had been shown by Mátyus to be a reactive dienophile in a Diels-Alder reaction with dimethylbutadiene [19], was found to be a suitable reagent for this purpose in combination with the diene **1a** as well as, more importantly, with the dimethylpyranoindolone **1b**.

The cycloaddition is carried out by heating the reactants in 1,2,4-trichlorobenzene to 180-190 °C. The ratio of the isomeric cycloaddition products **11a,b** and **12a,b** shown in Scheme 4 strongly depends on the nature of the R group (H or CH_3). Employment of **1b** gave rise to the formation of two hexacyclic side products with a carbazolocarbazole structure (**13a,b**), which were isolated and characterized [17]. A possible mechanism for this side reaction is shown in Scheme 5.

When a 2-benzyl-substituted 5-ethanesulfonylpyridazinone was reacted with **1b** under the same conditions (heating in 1,2,4-trichlorobenzene), a mixture of carbazole-fused *N*-benzylpyridazinones was obtained in moderate yield. Debenzylation with aluminium trichloride led to the key intermediates **15a,b** which on treatment with phosphorus oxychloride gave the chloro compounds **16a,b**. Finally, reductive dechlorination afforded the target 3-aza-*ellipticine* **17** [20].

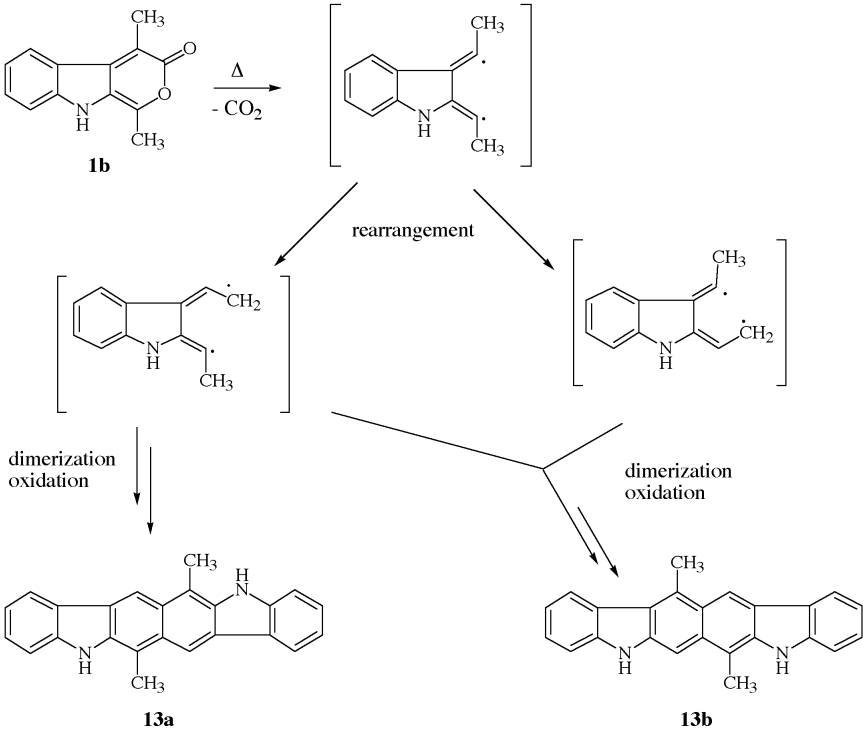
A more efficient route to compounds of type **15** was elaborated, starting from the lactones **10a,b** (compare Scheme 3). These compounds had been obtained by pyridinium chlorochromate oxidation of the dialcohol **9**, but were found to be more conveniently available by Diels-Alder reaction of **1b** with a tetrahydropyranyl-protected hydroxybutynoic acid ester. *N*-Protection with a benzenesulfonyl moiety, followed by selective bromination of the CH_2 group with *N*-bromosuccinimide/dibenzoyl peroxide gives the intermediate bromo lactone **19**, which on refluxing in hydrazine hydrate undergoes pyridazine ring closure as well as *N*-deprotection to afford the required pyridazinone.

Scheme 4

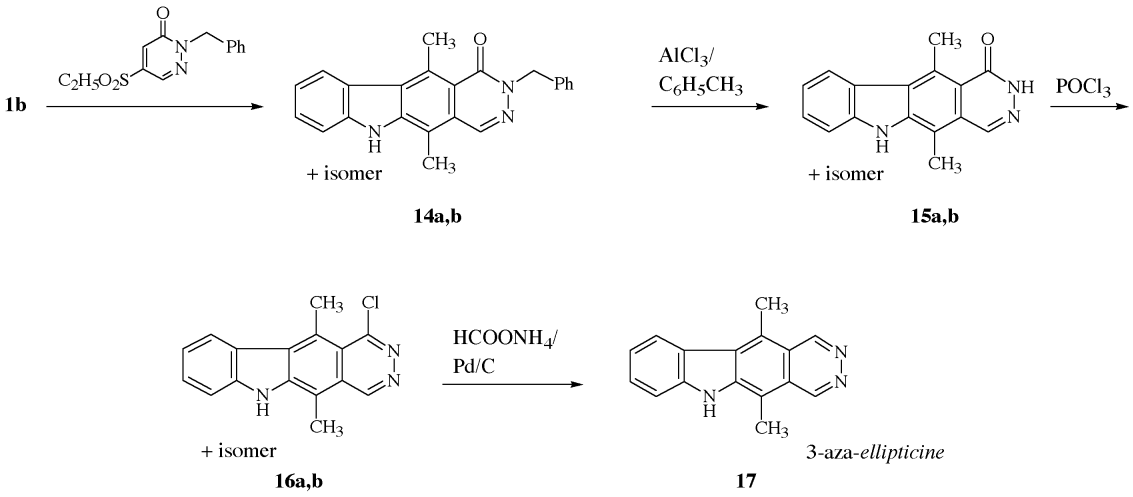


	ratio	
11a R = H	3 : 1	**11b** R = H
12a R = CH_3	1 : 3	**12b** R = CH_3

Scheme 5



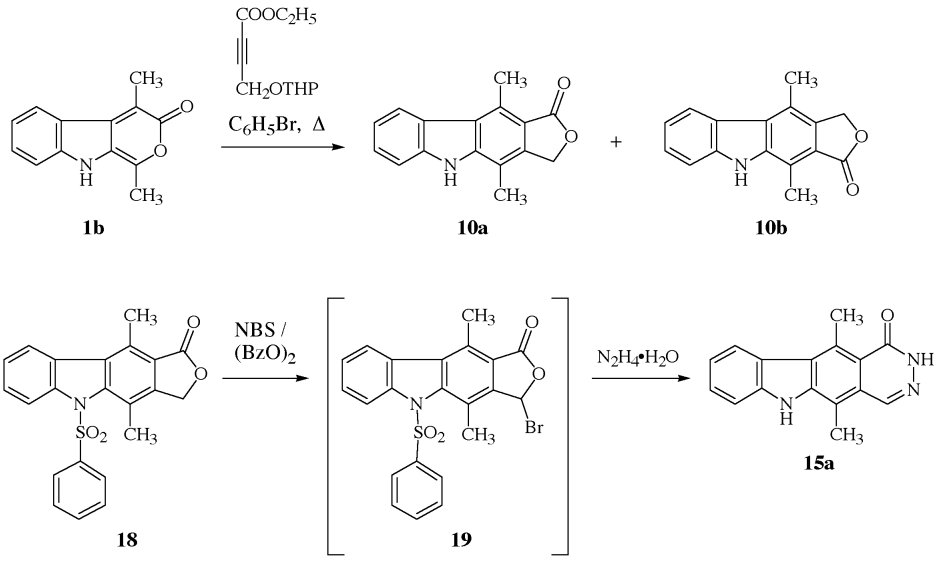
Scheme 6



Synthesis of 3-Aza-*Olivacine*.

In a similar manner as described above, starting from the monosubstituted diene **1a**, a synthetic pathway to the novel 3-aza isoster (**30**) of the antitumor alkaloid *olivacine* was developed [21]. In a first approach, after some unsuccessful experiments with the corresponding anhydride, we used the protected *N*-phenyl imide **20** as the key intermediate, which is available in good yields from the diester. For steric reasons, attack of methyl lithium could be expected to

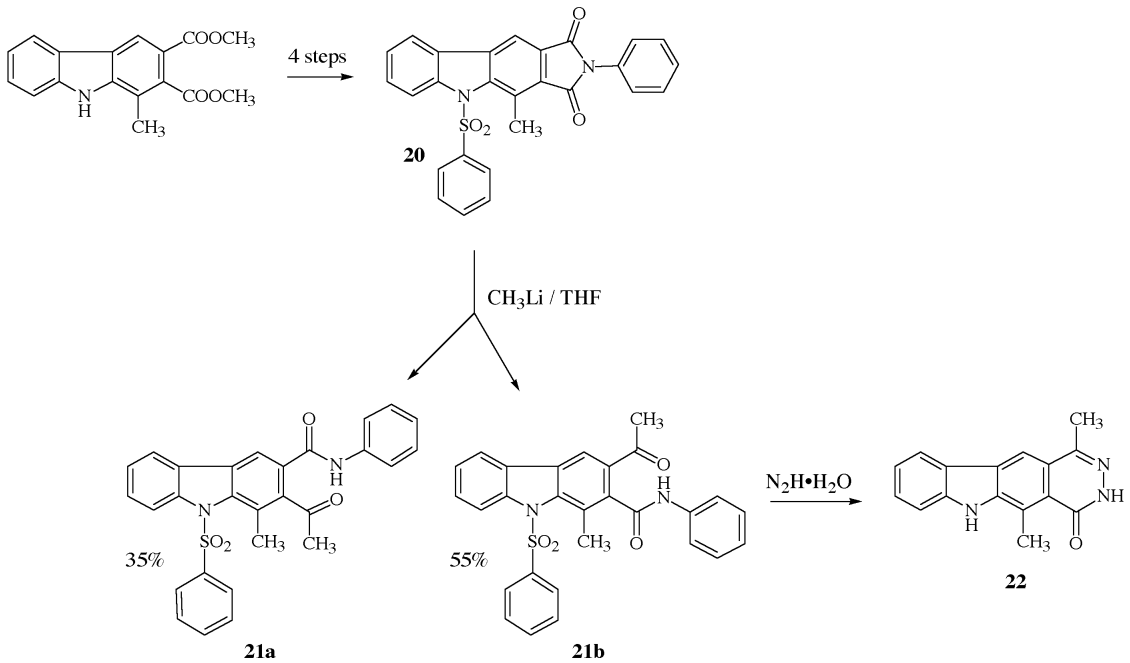
Scheme 7



occur preferentially at C-1 rather than at C-3 of the imide structure. This is the case indeed, however, the regioselectivity of this introduction of the required methyl group was disappointing (isomer ratio **21a**:**21b** = 35:55) and, moreover, the chromatographic separation of the mixture turned out to be difficult. Refluxing of the 3-acetyl compound (**21b**) in excess hydrazine hydrate then gave the deprotected carbazole-fused pyridazinone **22**, from which the target 3-aza-*olivacine* should be accessible in only two further steps.

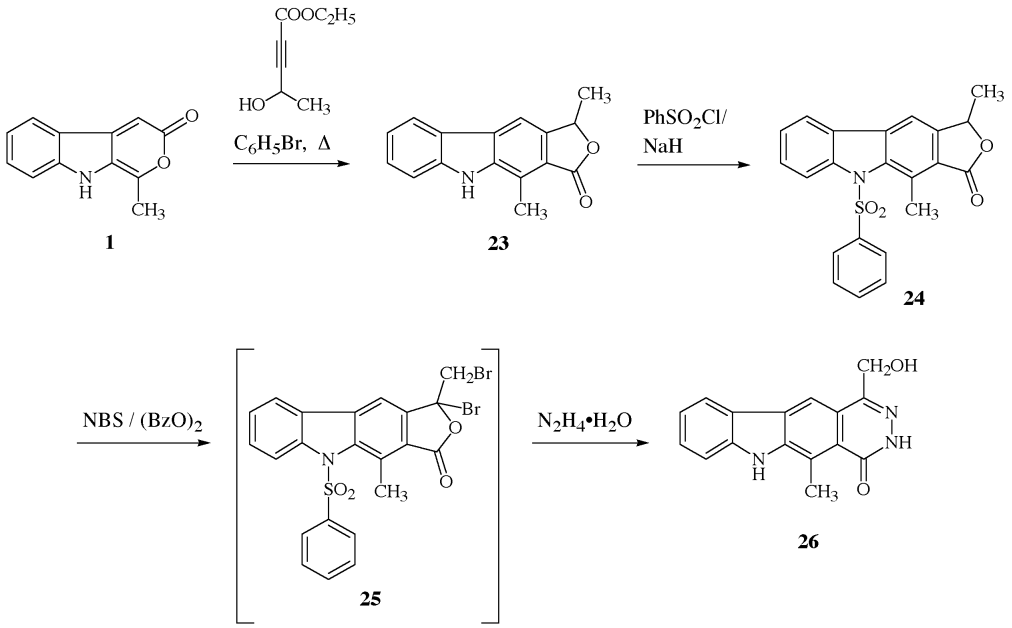
In view of the difficult isomer separation, we decided to investigate alternative routes to the pyridazinone **22** by introducing all of the required carbon atoms already at the stage of the initial Diels-Alder reaction with the fused

Scheme 8



pyrone **1a**. Thus, based on previous findings by Moody and Shah [22], the pyrone could be transformed into the lactone **23** regiospecifically and in good yield, by using the appropriate hydroxypentynoic acid ester as a dienophile. The reaction is carried out in bromobenzene at 150 °C. In order to introduce the necessary ketone functionality, we attempted to apply the same bromination method as we had used before in the *ellipticine* series (compare Scheme 7), using NBS and dibenzoyl peroxide, after the carbazole nitrogen had been protected with a benzenesulfonyl group. However, in this case, we were not able to obtain the desired bromo lactone: treatment of compound **24** with one equivalent of NBS gave a 1:1 mixture of unreacted starting material and a compound which must be a dibrominated derivative (**25**). The yield of this dibromo compound could be raised to about 80% by employment of two equivalents of the brominating agent. This unstable intermediate could not be isolated and purified, however it could be easily transformed into a stable product, again by prolonged refluxing in hydrazine hydrate. Under these conditions, the protecting group is split off and the pyridazinone ring is formed, affording the hydroxymethyl-substituted compound **26**.

Scheme 9

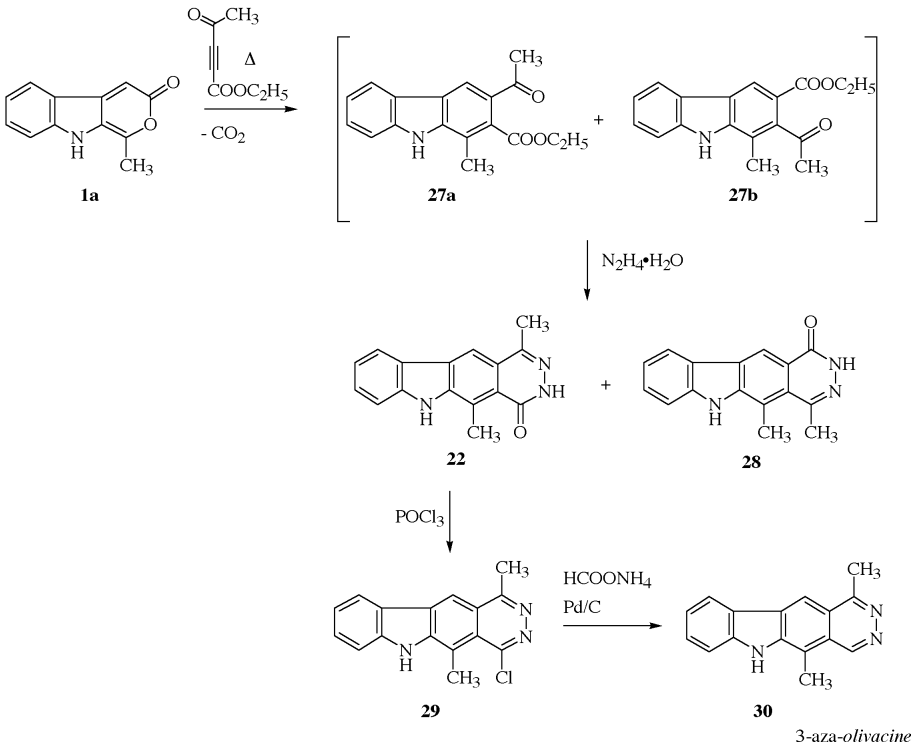


In order to circumvent this problem, a modification of this pathway was envisaged which should avoid the need to oxidize the lactone CH substructure. This was accomplished by using ethyl 4-oxopent-2-ynoate as a dienophile in the Diels-Alder reaction. In this case however, the cycloaddition reaction shows very little regioselectivity, which had to be expected, in view of Moody's previous observations with a similar reagent [22]. The ratio of the isomeric keto esters **27a** and **27b** is about 1.2 to 1 in favor of the desired isomer (**27a**). On the other hand, the combined yield is very high and the reaction proceeds very smoothly within 2 hours of refluxing in bromobenzene. The esters **27a,b** could not be separated, but refluxing of the mixture in ethanolic hydrazine hydrate afforded the pyridazinone **22** in a very fast reaction (15 minutes), whereas the analogous cyclization of **27b** into **28** takes several hours. Thus, the key intermediate **22** could be isolated very easily and in an acceptable yield. Conversion of the pyridazinone **22** into the chloro compound **29** and subsequent hydrogenolysis finally leads to the *olivacine* analog **30** (Scheme 10). In a preliminary *in vitro* screening, compound **30** showed 40-90% growth inhibition towards four different tumor cell lines at a concentration of 3.16 μg/ml.

Synthesis of 5,6-Bridged Pyridazino[4,5-*b*]carbazoles.

The most recent extension of our investigations in the field of pyridazine-fused carbazoles aims at the construction of pentacyclic compounds, in which the 5-methyl group is incorporated into a propylene unit linking C-5 and N-6 of the pyridazino[4,5-*b*]carbazole skeleton. For the construction of such systems, an entirely different strategy than that discussed above was employed [23], featuring an intramolecular inverse-electron-demand Diels-Alder reaction as the key step (Scheme 11). The indole moiety as an electron-rich dienophile [24] and the pyridazine as the electron-deficient diene should be tethered by a propylene chain, which we intended to

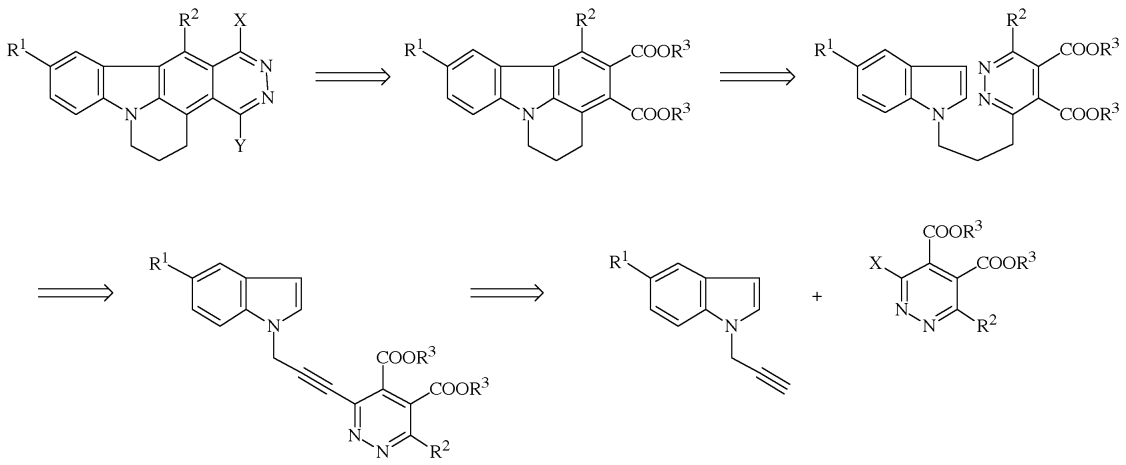
Scheme 10



construct by a coupling reaction of a terminal acetylene with an appropriate halopyridazine, followed by catalytic hydrogenation of the triple bond.

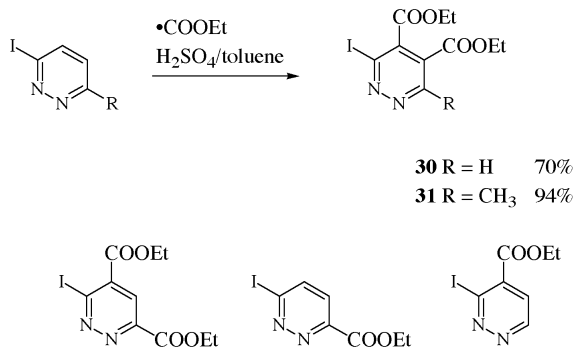
Following this concept, we developed a synthesis for the requisite 3-iodo-4,5-pyridazinediesters by radicalic ethoxycarbonylation of 3-iodopyridazine and its 6-methyl derivative, respectively [25]. This type of direct introduction of ester groups into π-deficient heteroaromatics had been extensively studied by Minisci [26] and was later optimized by Heinisch and Lötsch [27], who proposed a two-phase solvent system in order to suppress polysubstitution. Whereas Dal Piaz had successfully converted 3-chloro-6-methylpyridazine into the corresponding 4,5-diester [28], there have been no reports so far dealing with iodopyridazines or other iodo-substituted heteroaromatics in this reaction type. The ester radicals are easily generated by Fe^{2+}-induced redox decomposition of the oxyhydroperoxide formed from ethyl pyruvate and hydrogen peroxide. In our case, the reaction works best in a two-phase system of sulfuric acid and toluene under -

Scheme 11



ice-cooling and gives a yield of 94% of the methyl-substituted iodo diester (**31**) and about 70% for the 6-unsubstituted analog (**30**). With the latter educt, we observed the formation of small amounts of side products, which are shown at the bottom of Scheme 12.

Scheme 12

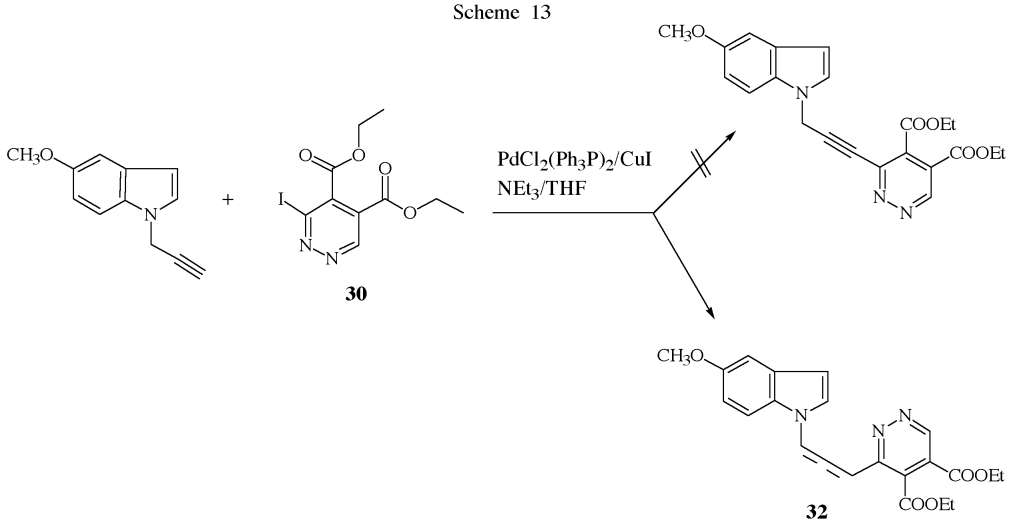


Whereas the *Sonogashira* coupling reaction of 5-methoxy-*N*-propargylindole and 3-iodopyridazine or 1-iodophthalazine smoothly afforded the expected alkynyl-substituted 1,2-diazines, employment of the iodo ester **30** under identical conditions led to the formation of a rearranged product, *i.e.*, the allene **32**. This compound turned out to be very stable and the allene structure could not be hydrogenated.

the ester functionalities were required for the following reaction steps, we attempted to avoid this rearrangement by employing an indole precursor with a significantly higher electron density at the nitrogen atom. Indeed, the Pd-catalyzed cross-coupling reaction of **30**,**31** was successfully accomplished, using propargyl-substituted indolines as the alkyne components (Scheme 14), thus affording compounds **33a,b** and **34a,b** in reasonable yields. The triple bond in these structures could be easily transformed into the required single bond by catalytic hydrogenation. However, under these conditions also the electron-poor 1,2-diazine nucleus was partially reduced to give dihydropyridazines of type **35**,**36**. Although this was not expected, it presented no serious problem, as a subsequent dehydrogenation step was necessary in any case, in order to convert the indoline structure into an aromatic indole unit. Thus, compounds **35** and **36** were refluxed with palladium on carbon in xylene solution in the presence of air, so that both the diene and the dienophile units (*i.e.*, the electron-deficient pyridazine and the electron-rich indole) were generated in a single step.

With the successful synthesis of compounds **37a,b** and **38a,b**, the stage was set for the intramolecular inverse-electron-demand Diels-Alder reaction, which was the key step in the envisaged pathway to 5,6-bridged 3-aza-*ellipticines*. This thermally induced [4+2] cycloaddition reaction was accomplished by refluxing the educts in 1,3,5-triisopropylbenzene solution under careful exclusion of air. After work-up, the aromatic carbazoles **39**,**40** were obtained which are formed by oxidation of the initial

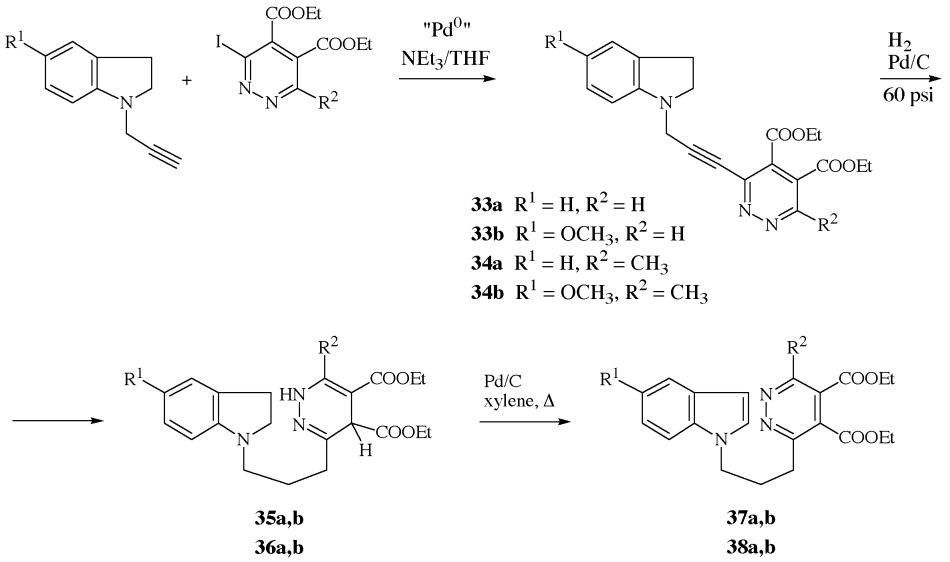
Scheme 13



As the reason for the observed alkyne-allene rearrangement, the relatively high acidity of the NCH_2 substructure, as a result of the electron-withdrawing effect of the ester groups attached to the pyridazine ring, can be assumed. As

dihydro adducts (Scheme 15). With the 6-unsubstituted pyridazines (**37a,b**), the yields are slightly higher and the cycloaddition is faster, compared to the 6-methyl analogs (**38a,b**) which are sterically more hindered. On the other

Scheme 14

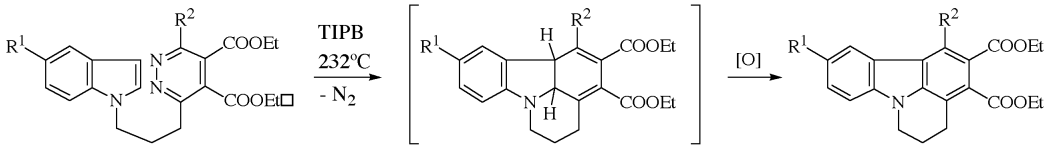


33a $R^1 = H$, $R^2 = H$
33b $R^1 = OCH_3$, $R^2 = H$
34a $R^1 = H$, $R^2 = CH_3$
34b $R^1 = OCH_3$, $R^2 = CH_3$

35a,b
36a,b

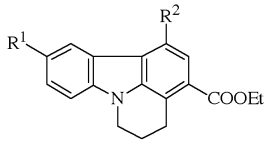
37a,b
38a,b

Scheme 15



37a,b
38a,b

39a,b
40a,b



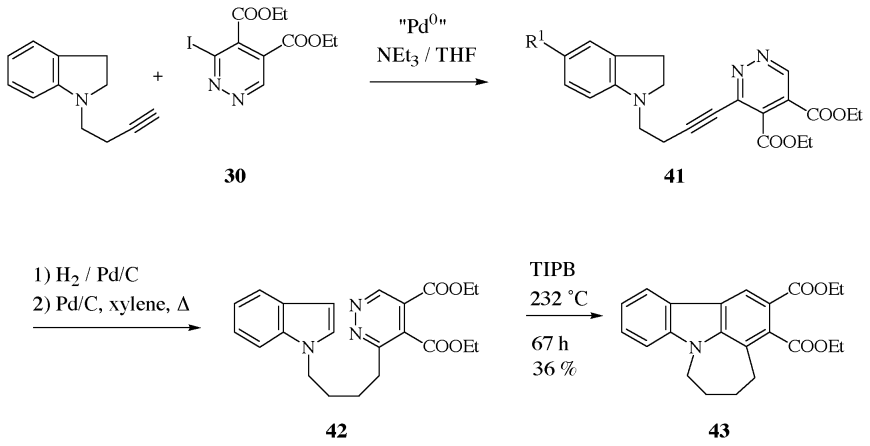
	$R^1 = H$	$R^1 = OCH_3$
$R^2 = H$	17 h 55%	17 h 51%
$R^2 = CH_3$	50 h 43%	50 h 41%

hand, the presence or absence of an electron-donating methoxy group at the indole ring makes no big difference with respect to yields and reaction times. Interestingly, in all cases we found also small amounts of a side product in which one of the ester groups is lost.

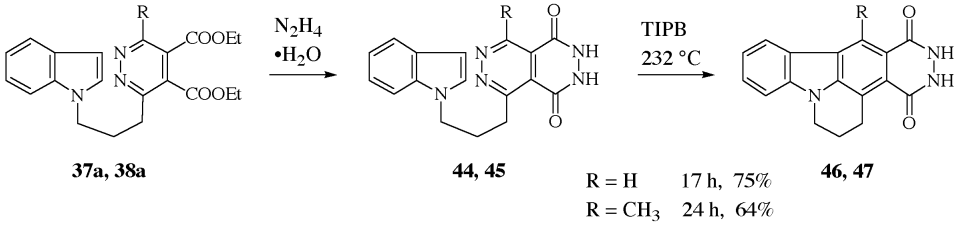
As demonstrated by the sequence displayed in Scheme 16, this intramolecular cycloaddition reaction also works when the chain length of the tether is increased by one methylene unit, although the yield is somewhat lower and the required reaction time is longer, because of the lower degree of entropic assistance which is associated with this longer linking unit.

When the fused carbazolediesters of type **39** and **40** were refluxed in hydrazine hydrate, we obtained the corresponding pyridazinedione only from the compound with $R^2 = H$ (**39**). The methyl-substituted analog (**40**) afforded an *N*-aminoimide, which was not surprising in view of our previous observations (compare Scheme 3). This problem could be solved by reverting the order of the two steps, cycloaddition and condensation with hydrazine. Reaction of mono- as well as disubstituted 4,5-pyridazinedicarboxylic acid esters (**37**,**38**) affords the pyridazine-fused pyridazinediones **44**,**45** without any side product, and moreover these compounds were found to undergo the intramolecular inverse Diels-Alder reaction in even shorter time and in better yields than the esters. Thus, the target ring system (compounds **46**,**47**) could be made available in reasonable overall yields. The functionalization of the pyridazine substructure of the pentacyclic skeleton is

Scheme 16



Scheme 17



the aim of our present investigations in order to synthesize a new series of *ellipticine* analogs with potential use as antineoplastic agents.

Acknowledgements.

I wish to acknowledge the skilful and enthusiastic contributions of all coworkers, students and collaborators whose names appear in the list of references. I also want to thank the National Cancer Institute (Bethesda, USA) and ASTA Medica (Frankfurt/Main, Germany) for performing the *in vitro* antitumor screenings.

REFERENCES AND NOTES

[1] S. Goodwin, A. F. Smith and E. C. Horning, *J. Am. Chem. Soc.*, **81**, 1903 (1959).

[2] G. W. Gribble in The Alkaloids, Vol. **39**, A. Brossi, ed, Academic Press, New York, 1990, p 239.

[3] M. Ohashi and T. Oki, *Exp. Opin. Ther. Patents*, **6**, 1285 (1996).

[4] C. Paoletti, J. B. Le Pecq, N. Dat-Xuong, P. Juret, H. Garnier, J. L. Amiel and J. Rouesse, *Recent Results Cancer Res.*, **74**, 107 (1980).

[5] C. Auclair, A. Pierre, E. Voisin, O. Pepin, S. Cros, C. Colas, J. M. Saucier, B. Verschuere, P. Gros and C. Paoletti, *Cancer Res.*, **47**, 6254 (1987).

[6] C. Ducrocq, F. Wendling, M. Tourbez-Perrin, C. Rivalle, P. Tambourin, F. Pochon, E. Bisagni and J. C. Chermann, *J. Med. Chem.*, **23**, 1212 (1980).

[7] M. J. Vilarem, J. Y. Charcosset, F. Primaux, M. P. Gras, F. Calvo and C. J. Larsen, *Cancer Res.*, **45**, 3906 (1985).

[8] K. W. Kohn, M. J. Waring, D. Glaubiger and C. A. Friedman, *Cancer Res.*, **35**, 71 (1975).

[9] C. Auclair and C. Paoletti, *J. Med. Chem.*, **24**, 289 (1981).

[10] S. Archer, B. S. Ross, L. Pica-Mattoccia and D. Cioli, *J. Med. Chem.*, **30**, 1204 (1987).

[11] Y. Pommier, *Cancer Chemother. Pharmacol.*, **32**, 103 (1993).

[12] M. Ohashi, E. Sugikawa and N. Nakanishi, *Jpn. J. Cancer Res.*, **86**, 819 (1995).

[13] H. Landelle, D. LaDuree, M. Cugnon de Sevricourt and M. Robba, *Chem. Pharm. Bull.*, **37**, 2679 (1989).

[14] N. Haider, R. Jbara, F. Khadami and R. Wanko, *Heterocycles*, **48**, 1609 (1998).

[15] C. J. Moody, *J. Chem. Soc., Perkin Trans. 1*, 2505 (1985).

[16] N. Haider and R. Wanko, *Molecules*, **4**, M105 (1999).

[17] N. Haider, J. Käferböck and P. Mátyus, *Heterocycles*, **51**, 2703 (1999).

[18] F. Fariña, M. V. Martín, M. Romañach and F. Sánchez, *An. Quím.*, 1988, **84-C**, 173.

[19] P. Mátyus, K. Fuji and K. Tanaka, *Heterocycles*, **36**, 1975 (1993).

[20] N. Haider, A. Hargitai and P. Mátyus, to be published.

[21] N. Haider and E. Sotelo, to be published.

[22] C. J. Moody and P. Shah, *J. Chem. Soc., Perkin Trans. 1*, 1407 (1988).

[23] N. Haider and J. Käferböck, to be published.

[24] For recent examples of intramolecular inverse-electron-demand Diels-Alder reactions of indole-linked 1,2,4,5-tetrazines and 1,2,4-triazines cf. [a] S. C. Benson, L. Lee, L. Yang and J. K. Snyder, *Tetrahedron*, **56**, 1165 (2000); [b] Z.-K. Wan and J. K. Snyder, *Tetrahedron Letters*, **39**, 2487 (1998); [c] W.-H. Fan, M. Parikh and J. K. Snyder, *Tetrahedron Letters*, **36**, 6591 (1995); [d] S. C. Benson, J.-H. Li and J. K. Snyder, *J. Org. Chem.*, **57**, 5285 (1992).

[25] N. Haider and J. Käferböck, *Heterocycles*, **53**, 2527 (2000).

[26a] F. Minisci, *Synthesis*, 1 (1973); [b] F. Minisci and O. Porta, *Adv. Heterocyclic Chem.*, **16**, 123 (1974); [c] F. Minisci, E. Vismara and F. Fontana, *Heterocycles*, **28**, 489 (1989).

[27a] G. Heinisch and G. Lötsch, *Tetrahedron*, **41**, 1199 (1985); [b] G. Heinisch and G. Lötsch, *Angew. Chem.*, **97**, 694 (1985).

[28] V. Dal Piaz, M. P. Giovannoni and G. Ciciani, *Tetrahedron Letters*, **34**, 3903 (1993).