Synthetic Methods

A Two-Step Synthesis of Cytostatically Active Benzo[*c*]phenanthridine Derivatives**

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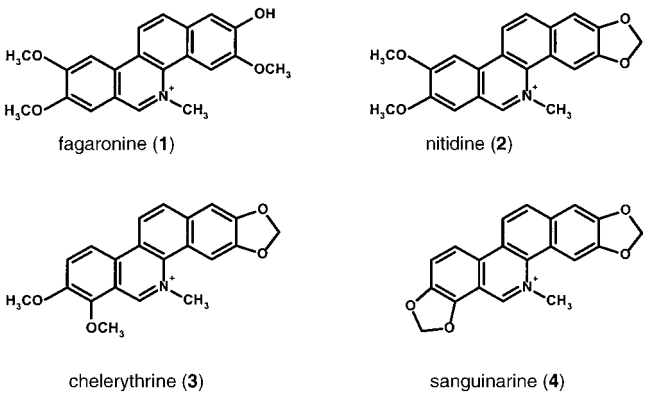
The benzo[*c*]phenanthridine class of substances display a variety of pharmacological properties. A large number of naturally occurring alkaloids that contain a benzo[*c*]phenanthridine ring system and have a known spectrum of activity are mentioned in the literature.[1,2] Aside from fagaronine (**1**), the most important representative of this group of natural products, other alkaloids with extensive pharmacological potential are nitidine (**2**), chelerythrine (**3**), and sanguinarine (**4**; Scheme 1).[3–5] The synthesis of these natural products is of great interest, because they can be isolated from plant materials only in very small amounts. Cushman et al.[6] describe yields in the 0.003 to 0.07% range for the isolation of **2** from a series of zanthoxylum and fagara varieties. Compound **1** was first isolated from the root of *Fagara zanthoxyloides* in 1972.[7]



Scheme 1. Structural formulas of known benzo[*c*]phenanthridine alkaloids.

The first total synthesis of **1** was described by Gillespie et al. in 1974.[8] This synthetic route gave **1** in 0.7% yield starting from 2,3-dimethoxy-5-nitronaphthalene. Other synthetic routes to **1** have been described by Ishii et al.,[9] Treus et al.,[4] Lunch et al.,[5]and Šmidrkal[10], the latter being only a slight variation on the synthetic sequence reported by Gillespie et al.[8].

Messmer et al.[7] demonstrated the pronounced in vivo antileukemia activity of **1**, which was then verified by Stermitz et al.[11] and in preclinical studies at the National Cancer Institute[12]. The pharmacological and toxicological effects of **1** and other alkaloids in this group have since been tested in many investigations. Sethi[13] and Kakiuchi et al.[14] have thus reported the inhibition of reverse transcriptases, which, as enzymes specific to tumor viruses, are responsible for the transcription of viral RNA into the complementary DNA. This DNA version of the viral genome is incorporated into the DNA of the host and is replicated in the cell-division process. Compound **1** additionally demonstrates DNA-polymerase-inhibiting properties, presumably a result of interaction with adenosine/thymine base pairs.[15]

In 1983 Pezzuto et al.[16] reported that the primary mechanism of the interaction of fagaronine with deoxyribonucleic acid is DNA intercalation. It was additionally demonstrated that binding of **1** is not—as described by Sethi[15]—limited to adenine- and thymine-containing polynucleotides. Furthermore, **1** has the ability to act as an inhibitor of topoisomerase I and II. In addition to restraining topomerase activity, this leads to a stabilization of the DNA–enzyme complex.[17]

Compound **2** exhibits a spectrum of activity similar to that of **1**.[18] As a result of the acute toxicity demonstrated by **2** in preclinical trials, its development as a pharmaceutical was not pursued.[19] Because the structures of **1** and **2** differ only by one methylenedioxy group, this is thought to be linked to the toxicity of **2**.[20]

Because of the great interest in the biological activity of these natural substances, the synthesis of benzo[*c*]phenanthridine derivatives and alkaloids in this family is an important area of heterocyclic chemistry. The toxicological problems that accompany some of the naturally occurring alkaloids have led to the development of new synthetic pathways for benzo[*c*]phenanthridine derivatives.[2,21,22] A variety of benzo[*c*]phenanthridine derivatives have thus become synthetically accessible.[23–26] However, these syntheses are very unwieldy and require many steps. In particular, compounds with substituents directly flanking the endocyclic nitrogen atom have thus far been very inconvenient to produce.[27,28]

By raising the reaction temperature when we attempted the synthesis of *E*-2-[2-(4-dimethylaminophenyl)vinyl]benzonitrile (**8**; Scheme 3) from 4-dimethylaminobenzaldehyde (**5**) and 2-methylbenzonitrile (**6**) as given in ref. [29], we surprisingly obtained **7a** as the main product (Scheme 2). Doubling the concentration of **6** used in the reaction increased the yield of **7a**, which is formed as a racemic mixture.

No synthesis of benzo[*c*]phenanthridines with phenyl substituents at C11 have thus far been described. In 1968 Devanathan et al.[30] reported attempting the synthesis of 4*b*,10*b*,11,12-tetrahydro-11-phenylbenzo[*c*]phenanthridine. The synthesis failed in the cyclization of the acetamido or benzamido derivative of 2,3-diphenyl-1,2,3,4-tetrahydrobenzo-1-naphthylamine mediated by phosphorus oxychloride or polyphosphoric acid to form the corresponding benzo[*c*]-

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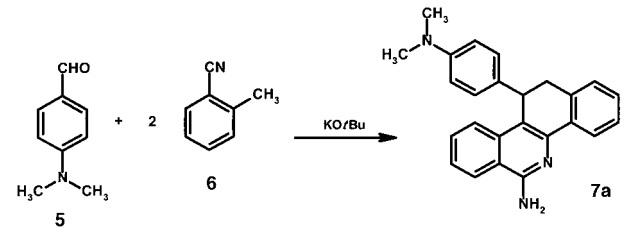


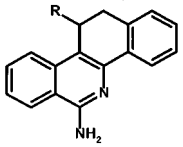
Scheme 2. Synthesis of **7a**.

phenanthridine. The synthesis of **7a** from **5** and **6** reveals a new and surprisingly simple synthetic route for the production of benzo[*c*]phenanthridine derivatives. The direct reaction of **8** with **6** under the conditions described above also leads to **7a**. We can thus assume that the formation of **7a** proceeds by formation of intermediate **8** (Scheme 3).

We view the following to be a probable reaction pathway. In analogy to a vinylogous Michael reaction, a further equivalent of **6** adds to the double bond in **8**. The negative mesomeric and inductive effect of the nitrile group facilitates the nucleophilic addition. Protonation could lead to **9**; however, in the presence of potassium *tert*-butanolate the carbanion **10** is very likely to be present. The intramolecular nucleophilic addition of this carbanion to the nitrile group in the manner of a Thorpe–Ziegler reaction then leads to the tautomeric phenylogous enaminonitrile **12** by way of the iminonitrile **11**. According to investigations carried out by Baldwin,[31] the enaminonitrile **12** should be favored over iminonitrile **11**. Nitrile functionalities in enaminonitriles are activated toward nucleophilic attack by the formation of mesomeric structures.[31,32] In this way, and also supported by the steric arrangement, the amino group of **12** adds intramolecularly to the nitrile group, resulting in **13**. Subsequent tautomerization finally leads to **7a**.

The route used for the formation of **7a** was also used to obtain other benzo[*c*]phenanthridine derivatives (Table 1).

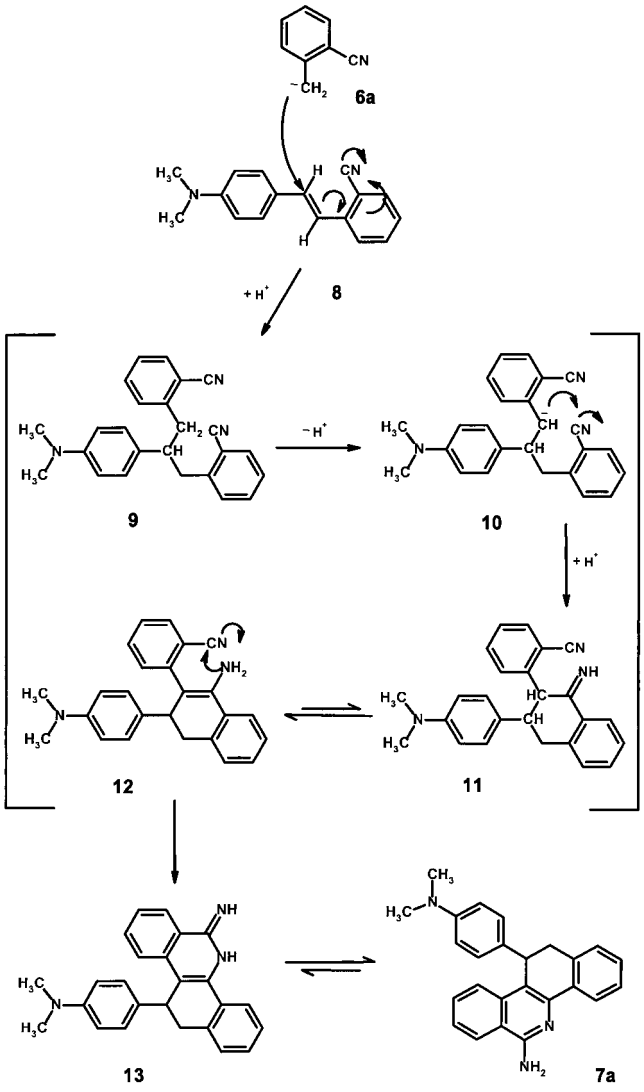
Table 1: Synthesis of 6-amino-11,12-dihydrobenzo[*c*]phenanthridines.



R	Product	Yield [%]
4-$Me_2NC_6H_4$	**7a**	40
Ph	**7b**	37
4-$MeOC_6H_4$	**7c**	53
2,4-$(MeO)_2C_6H_3$	**7d**	37
3,4-$(MeO)_2C_6H_3$	**7e**	57
H	**7f**	11

As demonstrated by the successful use of benzaldehyde, an electron-donating group is not necessary for sufficient polarization of the double bond. The phenyl derivative **7b** was obtained in similar yields to **7a**. Replacement of the dimethylamino group by a methoxy group led to higher product yields (**7c**). The dimethoxy derivative **7d** was obtained in lower yields, probably because of steric hindrance. In contrast, the dimethoxy derivative **7e** was obtained in yields similar to those of **7c**. The use of formaldehyde as the aldehyde component resulted in a lower yield (**7f**); the reasons for this are unclear.

Dehydration with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)[33] converted the dihydro compounds into the corresponding 6-aminobenzo[*c*]phenanthridines in yields ranging from 14 to 38% (Scheme 4). These results contradict previous findings,[24] in which 11,12-dihydrobenzo[*c*]phenanthridine and a series of substituted 11,12-dihydrobenzo[*c*]-phenanthridines could be dehydrated only by heating in the presence of 30% palladium over activated carbon. Heating

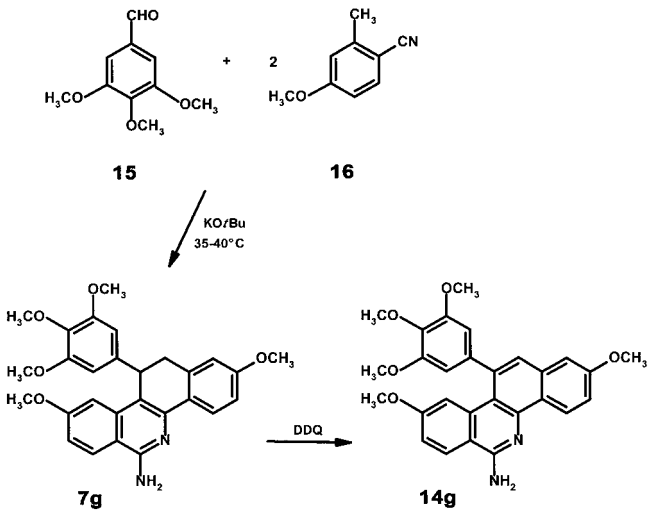


Scheme 3. Postulated mechanism for the formation of **7a**.

Scheme 4. Dehydration of **7** to form **14**.

with DDQ in various solvents or heating with 10% palladium over activated carbon did not lead to the desired products. In preliminary experiments, the hydrochlorides of **7** could also be dehydrated by heating with sulfur.[34] However, this method demonstrated no advantages over dehydration with DDQ, as the yields were lower. As described above,[24] attempts at dehydration with 10% palladium over activated carbon were not successful.

Since many natural products have substituents in positions 2 and 3, or 8 and 9, we were interested determining whether the method described above could be used to prepare derivatives having substituents in these positions directly. For this, substituted 2-methylbenzonitriles are necessary. Whereas electron-withdrawing groups should not be problematic, electron-donating groups could have an adverse effect on both the deprotonation of the methyl groups in the *ortho* position and on the nucleophilic addition. The example of easily accessible 4-methoxy-2-methylbenzonitrile[35] and its conversion with 3,4,5-trimethoxybenzaldehyde to **7g** demonstrates the broad applicability of this synthetic route (Scheme 5). At 71%, the yield was even surprisingly high. The 24% overall yield obtained after dehydration of **14g** was within the same range as the other conversions.



Scheme 5. Synthesis of **7g** and **14g**.

The products obtained can be converted into other benzo[*c*]phenanthridine derivatives, especially by substitution in the 6-position. In particular, removal of the amino group from **14f** should make the basic ring system of this class of compounds easily accessible. The use of 4,5-dimethoxy-2-methylbenzonitrile and paraformaldehyde should allow for the straightforward formation of a fagaronine precursor.

The yields of **7** and **14** are not outstanding; however, the substances can be prepared in large amounts and with analytical purity (for pharmacological studies, for example), without chromatographic purification. Initial investigations of compounds of type **14** and to some extent type **7** demonstrated a very high cytotoxic potential. Studies of **7g** and **14g** by the U.S. National Cancer Institute (NCI) serve to illustrate this. NCI uses in vitro cell line screening to test compounds for activity against 60 human tumor cell lines from nine different types of cancer.[36] For each compound tested, 60 dose–activity curves, corresponding to the number of tumor cell lines, with five test concentrations each are obtained. This makes it possible to obtain information about the cytotoxicity as well as the selectivity of each compound against one or more forms of cancer. In order to characterize the antitumor activity of a tested compound, the dose-dependent parameters GI_{50} (growth inhibition 50%), TGI (total growth inhibition), and LC_{50} (lethal concentration 50%) are calculated. For each of these three parameters, the meangraph midpoint (MG_MID) is also calculated; this is the mean of the logarithms of the GI_{50} values and corresponds to an average response characteristic of all 60 cell lines to the test substance. This factor allows for the characterization of the activity of a compound in the test system and for its quantitative comparison with other compounds. Taking the antilogarithm of this value gives the mean of the GI_{50} values over all cell lines.[37]

According to these studies, a 3.39 μM concentration of **7g** and a 0.18 μM concentration of **7e** halve the growth rate of the tumor cells. Both compounds thus demonstrate a higher activity than fagaronine (**1;** 9.48 μM). In addition to these data, Table 2 contains data for other cytostatically active compounds. With the exception of paclitaxel, **7e** and **7g** demonstrate better cytostatic activity than established cystostatics such as cyclophosphamide in the in vitro cell line testing of the NCI. Further benzo[*c*]phenanthridine derivatives (not presented here) demonstrated similar cytostatic activities. These results will thus initiate the further development of new potential cancer drugs.

Table 2: Cytotoxic activity of benzo[*c*]phenanthridine derivatives and selected cytostatics from in vitro cell line screening by the NCI.[12]

Name	MG_MID (GI_{50})	$\frac{\sum GI_{50}}{60}$ [μM]
7g	−5.47	3.39
7e	−6.74	0.18
fagaronine	−5.00	9.48
cyclophosphamide	−3.68	210
5-fluorouracil	−4.75	17.6
6-sulfanylpurine	−5.13	7.36
paclitaxel	−7.65	0.02

Experimental Section

7a–d: General synthetic protocol: A solution of the appropriate aldehyde (10 mmol) and 2-methylbenzonitrile (2.34 g, 20 mmol) in 5 mL 1,3-dimethyltetrahydro-2-pyrimidone (DMPU) was added dropwise under nitrogen to a solution of potassium *tert*-butanolate (2.47 g, 22 mmol) in 20 mL DMPU. The reaction mixture was stirred under nitrogen for 5 h at 35°C, then poured into a solution of ammonium chloride (2.2 g, 40 mmol) in 100 mL ice water, and extracted three times with 100 mL dichloromethane. The organic phase was filtered through cotton if necessary and concentrated to a volume of about 100 mL on a rotary evaporator. The organic phase was vigorously shaken with 3N hydrochloric acid; the resulting precipitate was filtered, washed with dichloromethane, and dried. The corresponding 11-substituted 6-amino-11,12-dihydro-benzo[*c*]phe-

nanthridinium chlorides were obtained after recrystallization. The free amines were released in diethyl ether with dilute ammonia. Addition of petroleum ether and removal of the diethyl ether on a rotary evaporator provided the solid products.

7e: As described above, with the following changes: 9.86 g (88 mmol) potassium *tert*-butanolate in 90 mL DMPU; 6.65 g (40 mmol) 3,4-dimethoxybenzaldehyde, 9.36 g (80 mmol) 2-methylbenzonitrile in 40 mL DMPU; dropwise addition at 40 °C; stirring for 4 h at 35–40 °C; 8.8 g (80 mmol) ammonium chloride in 400 mL ice water; extracted three time with 150 mL dichlormethane; vigorously stirred overnight with 20 mL 5 N hydrochloric acid.

7f: As described above, with the following changes: 2.47 g (22 mmol) potassium *tert*-butanolate in 20 mL DMPU; 0.3 g (10 mmol) paraformaldehyde; 2.34 g (20 mmol) 2-methylbenzonitrile in 12 mL DMPU; dropwise addition in 2 mL portions every 15 minutes; stirring for 6 h at 35 °C. The organic phase was concentrated on a rotary evaporator until vigorous precipitation occurred and was then stored overnight in a refrigerator; recrystallization from methanol/dichloromethane.

7g: A solution of 4-methoxy-2-methylbenzonitrile (8.83 g, 60 mmol) and 3,4,5-trimethoxybenzaldehyde (5.89 g, 30 mmol) in 30 mL DMPU was added dropwise under nitrogen to a solution of potassium *tert*-butanolate (7.41 g, 66 mmol) in 70 mL DMPU at 30 °C. The reaction mixture was subsequently stirred for 4 h at 35–40 °C and then carefully hydrolyzed in a solution of ammonium chloride (6.54 g, 120 mmol) in 300 mL ice water. The aqueous phase was extracted three times with 150 mL dichloromethane, and the combined organic phases were then dried over sodium sulfate and concentrated on a rotary evaporator. Precipitation of **7g** occurred after the organic phase had been stirred vigorously overnight with 10 mL concentrated hydrochloric acid and 10 mL water. The precipitate was filtered out, washed with a small amount of dichloromethane, recrystallized from methanol, and dried for 24 h under vacuum (oil pump).

14b–g: General synthetic protocol: A solution of DDQ in dioxane was added to a solution of **7a–g** in dioxane and heated to reflux. The cooled reaction mixture was hydrolyzed by pouring it into a saturated sodium hydrogen carbonate solution, and the reaction mixture was then extracted with diethyl ether. The ether phase was washed once with dilute sodium hydrogen carbonate solution and three times with water. The organic phase was dried over sodium sulfate and concentrated on a rotary evaporator. The 6-aminobenzo[*c*]phenanthridiniumperchlorate was precipitated by vigorous stirring of the organic phase overnight with 70% perchloric acid. The precipitate was filtered out and recrystallized from methanol. **14b**: 0.5 g (1.6 mmol) **7b** in 25 mL dioxane, 0.63 (2.8 mmol) DDQ in 25 mL dioxane, 4 h reflux; **14c**: 1.5 g (4.3 mmol) **7c** in 100 mL dioxane, 3.8 g (16.7 mmol) DDQ in 100 mL dioxane, 8 h reflux; **14d**: 0.5 g (1.3 mmol) **7d** in 10 mL dioxane, 0.54 g (2.3 mmol) DDQ in 35 mL dioxane, 4 h reflux; **14e**: 2.0 g (5.2 mmol) **7e** in 50 mL dioxane, 4.4 g (21.0 mmol) DDQ in 100 mL dioxane, 16 h reflux; **14f**: 0.25 g (1.0 mmol) **7f** in 15 mL dioxane, 0.40 (1.7 mmol) DDQ in 35 mL dioxane, 4 h reflux; **14g**: 1.62 g (3.4 mmol) **7g** in 90 mL dioxane, 3.11 g (13.7 mmol) DDQ in 70 mL dioxane, 9 h reflux.

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