(–)-(*M*,7*S*)-Colchicine and (–)-(*M*,7*S*)-10-Ethylthiocolchicide/Alkyne Cycloaddition Reactions: Synthesis of Novel Colchicine Derivatives by Consecutive [4+2] and [3+2] Cycloadditions☆

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Cycloaddition reactions of the facially dissymmetric diene moiety of (–)-(*M*,7*S*)-colchicine (**5**) and (–)-(*M*,7*S*)-10-ethylthiocolchicide (**9**) to various alkynes have been studied. With **5** and the dienophilic benzyne (**3**), dimethyl acetylenedicarboxylate (DMAD) (**4**) and cyclooctyne (**6**) as starting materials all cycloadditions could be realized with high regioselectivity at the 8,12-positions of the alkaloid. The approach of the dienophiles preferentially occurred toward the *syn* π-face of the diene. In contrast to the cycloaddition mode of **5** the ethylthiocolchicide **9** surprisingly reacted in a different manner. With benzyne as starting material a novel [3+2] cycloaddition of the thioenol ether moiety of **9** towards the dipolarophilic benzyne is supposed, affording the unexpected colchicide **10** after [1,5]H shift of the primarily formed cycloadduct followed by cleavage of the C–S linkage. With DMAD (**4**) and cyclooctyne (**6**) the reaction course is more complex. In a consecutive [4+2]/[3+2] cycloaddition (or vice versa) followed by a thermally induced cycloreversion of a not identified intermediate DMAD (**4**) gives rise to the polycyclic thiophene derivative **13** and the novel allocolchicinoid **14**. In a similar way cyclooctyne (**6**) yielded three products, the thiophene-annulated homobarrelenones **18** and **19** and the tetracyclic allocolchicinoid **21**. The structures of the novel colchicine derivatives were assigned on the basis of spectral data, those of the cycloadducts **1** and **19** were verified by X-ray crystallography. For the unprecedented formation of the various allocolchicinoids by consecutive [4+2]/[3+2] cycloadditions plausible reaction pathways are suggested, as far as possible. In addition the inhibitory effects on the tubulin polymerization reaction in vitro of **10**, **14**, and **21** are reported.

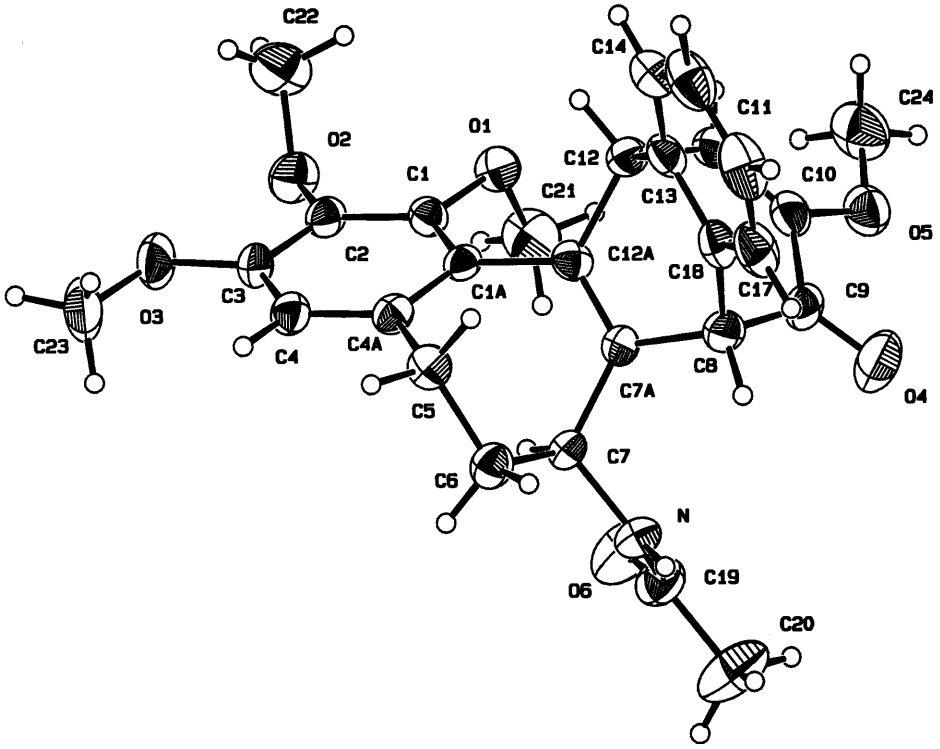
Our recent studies concerning $HOMO_{diene}/LUMO_{dienophile}$ controlled Diels-Alder reactions of (−)-(*M*,7*S*)-colchicine (**5**) have demonstrated that cycloaddition with several hetero- and carbodienophiles e.g. singlet oxygen, *N*-phenyl-1,2,4-triazoline-dione or *trans*-cyclooctene can be effected in good yields with high positional selectivity at the 8,12-positions of the alkaloid and preferentially from the diene face *syn* to the allylic substituent at the stereogenic center C-7[1]. In order to gain further insight into the steric and electronic factors that control [4+2] cycloadditions between the facially dissymmetric diene moiety of **5** and several highly reactive dienophiles on the one hand, and in view of the multifarious biological effects of the alkaloid and the possible pharmaceutical value of the cycloaddition products on the other hand, we have concentrated our synthetic efforts in extending these findings to [4+2] cycloadditions of the alkaloid **5** to several acyclic and cyclic alkynes; in addition we were interested to investigate the hitherto unknown diene properties of 10-ethylthiocolchicide (**9**) with the same dienophiles and to study their degree and sense of the positional and facial selectivity. Herein we disclose our recent findings in this area.

In all cases examined, cycloadditions of (−)-colchicine (**5**) with several alkynes were equally feasible, and the cycloadducts could be isolated in acceptable yields. Thus, when the alkaloid **5** was treated with benzyne (**3**) − prepared in situ from 1-aminobenzotriazole/lead tetraacetate in dry benzene[2] or from 2-phenyldiazonium carboxylate[3] − the exclusive formation of one single cycloadduct **1** (TLC analysis) was observed containing an interesting homobarrelenone moiety. After chromatography, the cycloadduct **1** could be isolated − depending on the starting material used − in 20% or 40% yield with 77% or 27% recovery of (−)-colchicine (**5**). The structure of the crystalline compound **1** was confirmed by mass, ^{1}H- and ^{13}C-NMR spectral analyses: Compared to the ^{1}H-NMR data of **5** the signals of 8-H and 12-H of **1** were shifted to higher field, while the ^{13}C-NMR signals of C-8 and C-12 were found at $\delta = 61.72$ and 48.18, respectively, verifying that cycloaddition had occurred regioselectively at the 8,12-positions of **5**. In analogy

  1434−193X/98/1111−2451 $ 17.50+.50/0

to the reaction mode of singlet oxygen[1] and other olefinic dienophiles we supposed that cycloaddition of benzyne (**3**) occurred again from the diene face syn to the allylic substituent at the stereogenic center C-7 to yield the *syn* cycloadduct **1**. This was conclusively proved by an X-ray crystallographic analysis (Figure 1).

Figure 1. ZORTEP plot of the structure of cycloadduct **1** in the crystal[4]; thermal ellipsoids at the 40% probability level, the numbering of the atoms differs from that given in the experimental section



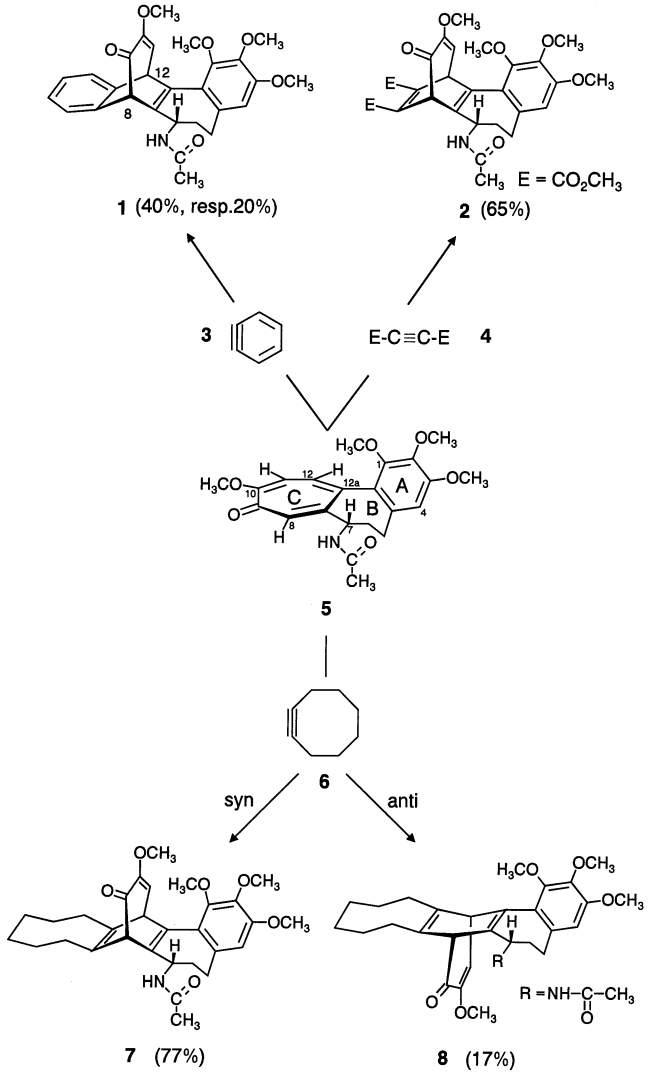
As already mentioned[1] refluxing a mixture of **5** and DMAD (**4**) in xylene at 140°C for 24 h resulted in the formation of two products, which were readily separable by column chromatography. The main product **2** was formed with high regio- and π-facial selectivity in 65% yield. Spectroscopic data (MS, ^{1}H and ^{13}C NMR) conclusively proved the expected structure of **2**, formed by cycloaddition of the alkyne **4** from the syn face to the NH group. It was accompanied by a small amount (ca. 1% yield) of another product, exhibiting the same parent ion $m/z = 541$, possibly the *anti* cycloadduct, the stereochemistry of which could not be elucidated.

Compared to *trans*-cyclooctene[1] cyclooctyne (**6**) is less reactive in the cycloaddition reaction with colchicine (**5**). Thus, the Diels-Alder reaction of **5** with **6** only proceeded under more drastic conditions to afford the expected cycloadducts. Refluxing a mixture of **5** and excess **6** in dry xylene for 24 h gave two products in 94% total yield, which could be separated by column chromatography, furnishing two stereoisomers with different polarity as derived from their R_f values. The more polar compound was isolated in 77%, the less polar in 17% yield. Because π-facial selectivity in [4+2] cycloadditions to the 8,12a-diene moiety of colchicine (**5**) is mainly controlled by steric factors[1], we suppose – consistent with the favored *syn*-facial selectivity observed for *trans*-cyclooctene – the more polar cycloadduct to be the *syn* product **7**, the less polar cycloadduct is assigned to be the *anti* product **8**, both again characterized by an intriguing homobarrelenone moiety.

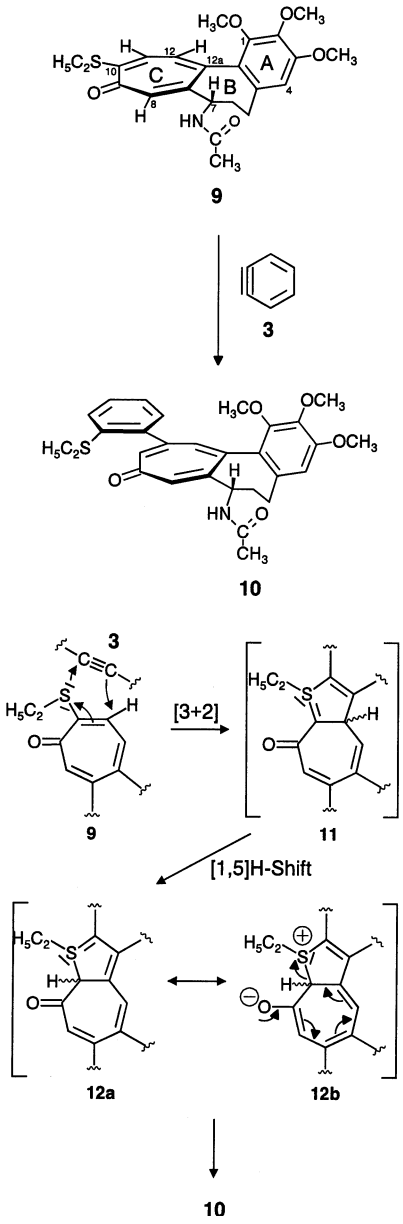
Having thus demonstrated that the diene (–)-colchicine (**5**) was effective in the "normal"-type Diels-Alder reaction with various alkynes, our attention was focused on hitherto unknown cycloaddition reactions with (–)-10-ethylthiocolchicide (**9**), easily accessible from **5** by treatment with ethanethiol in the presence of *p*-toluenesulfonic acid as a catalyst[5]. Surprisingly, the reaction of **9** with benzyne (**3**), performed under similar conditions as described above with **5**, produced an unexpected product in fair yield, identified as the colchicide derivative **10** by its spectroscopic properties (MS, ^{1}H- and ^{13}C-NMR). A comprehensible and plausible reaction sequence for the unprecedented formation of **10** is outlined in Scheme 2.

We suppose that in contrast to the cycloaddition behavior of **5** the first step of the reaction of the thiocolchicine **9** with **3** is not a [4+2] but an orbital-controlled [3+2] cycloaddition. In a hitherto unknown reaction mode the dipolarophile **3** reacts with the thioenol ether moiety of **9** which is characterized by 4 electrons in three parallel π-orbitals[6] like an allyl anion system, giving rise to the formation of the primary cycloadduct **11** with an annulated thiophene ring system. The question of a one- or two-step mechanism is undecided. Symmetry-allowed sigmatropic [1,5] hydrogen shift of **11** should lead to the intermediate **12**, which suffers a heterolytic cleavage of the C–S linkage as indicated yielding the unexpected colchicide **10**. To our knowledge this is the first example of a [3+2] cycloaddition

Scheme 1



Scheme 2

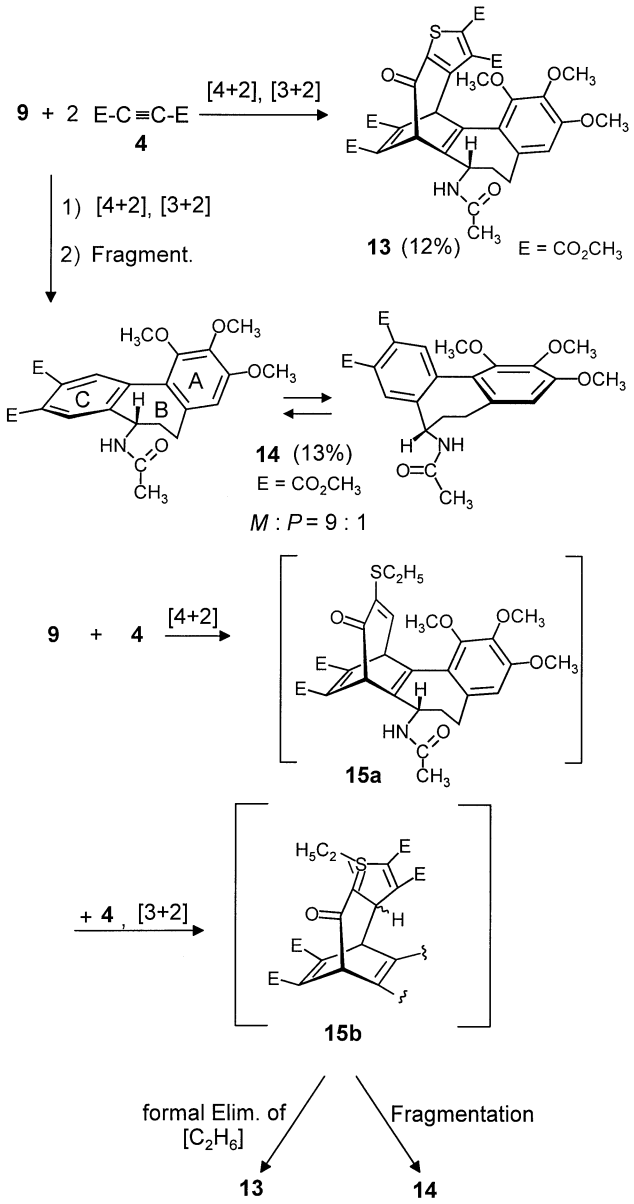


of a thioenol ether moiety with an alkyne and the observed reaction sequence is new and surprising.

Even more astonishing was the cycloaddition behavior of the thiocolchicine **9** towards DMAD (**4**). This electron-deficient alkyne proved sufficiently reactive providing fair yields of unexpected cycloadducts. Heating a solution of **9** with a fourfold excess of **4** in toluene at 130°C (pressure bottle) formed a complex mixture of products (TLC control). Careful, repeated silica gel column chromatography permitted the separation of two main products **13** (R_f = 0.26) and **14** (R_f = 0.20) in 25% total yield. The structural assignment of the pentacyclic thiophene derivative **13** obtained in 12% yield, is secured on the basis of the spectroscopic data (MS, ^{1}H and ^{13}C NMR). As is evident from the parent ion peak in the MS (*m/z* = 683) which indicated the molecular formula to be $C_{33}H_{33}NO_{13}S$, two molecules of **4** must have been combined with the thiocolchicine **9** under loss of one molecule of ethane. In addition detailed analyses of the ^{1}H- and ^{13}C-NMR data corroborate the assigned structure (see Experimental Section).

The same is true for the allocolchicinoid **14**, which in $CDCl_3$ solution exists as a mixture of two atropo-diastereomeric forms (*M*)-**14** and (*P*)-**14**, a phenomenon well known for instance for the novel alkaloid androbiphenyline[7d][7e]. Obviously, the ratio of the two atropodiastereomers [with (7*S*) configuration] in solution depends on the polarity of the solvent employed. This becomes evident from ^{1}H-NMR and possibly from optical rotation data. In the ^{1}H-NMR spectrum of **14** in $CDCl_3$ the two axially chiral conformers are seen in an (*M*)/(*P*) = 9:1 ratio. In CD_3OD solution only the (*M*) conformer could be detected, indicating that with the polarity of the solvent also the (*M*)/(*P*) ratio increases. Like most of the biologically active colchicine derivatives **14** exhibits strong negative specific rotation in $CHCl_3$ solution suggesting the (*M*) conformation of the bridged biaryl system with a counterclock-

Scheme 3



wise helicity predominating[1c]. Since ^{1}H-NMR data demonstrate that in CH_3OH the (*M*)/(*P*) ratio was increased it was obvious to suppose that the optical rotation should be shifted to a more negative value. This is the case. Since the specific rotational values measured are primarily associated with the skewed biaryl system the observed negative shift should be due to the atropoisomeric transformation of (*P*)-**14** to (*M*)-**14** although other effects cannot be excluded. Indicative for the conformational state [(*M*) or (*P*)] of the novel allocolchicinoid **14** is the chemical shift of the CH_3 protons of the *N*-acetyl group[7]. In the (*M*) conformation of both colchicine and isocolchicine the singlet of the *N*-acetyl protons ($CDCl_3$, at 500 MHz) is found at $\delta = 1.96$ and 2.06, respectively[7b]. In contrast to this finding the corresponding signal of the (*P*) conformation of isocolchicine appears at $\delta = 1.63$[7g]. Similar results have been obtained for the allocolchicinoid androbiphenyline, which has been determined to exist also as a mixture of two atropo-diastereomeric conformers in almost equal amounts[7e][7f]. In this case the protons of the *N*-acetyl group were detectable at $\delta = 1.98$ for the (*M*) form and at $\delta = 1.58$ for the (*P*) form. For the major atropisomer of **14** the signal of the *N*-acetyl protons appears at $\delta = 2.05$, for the minor at $\delta = 1.59$. It is therefore reasonable to assign the major conformer as possessing the (*M*) conformation and the minor conformer of **14** as existing in the (*P*) conformation.

Concerning the mechanism for the unprecedented formation of the thiophene annulated homobarrelenone **13** and the allocolchicinoid **14** (Scheme 3) we propose a reaction sequence including a consecutive [4+2]/[3+2] cycloaddition. This surprising reaction course is believed to start with a [4+2] cycloaddition of the first molecule of **4**. The Diels-Alder reaction occurs at the 8,12-positions of the alkaloid **9** with π-facial diastereoselectivity from the diene face *syn* to the *N*-acetyl group at the stereogenic center C-7[1] giving rise to the formation of the intermediate **15a**.

Subsequent [3+2] cycloaddition of the second molecule of **4** towards the thioenol ether moiety of **15a** should lead to the second intermediate **15b**, which is supposed to react in two directions under the drastic reaction conditions employed (xylene, 130°C). The formal elimination of $[C_2H_6]$ gives rise to the thiophene annulated homobarrelenone **13**, whereas the fragmentation with loss of a $C_{11}H_{12}O_5S$ moiety (not isolated) leads to the axially chiral biphenyl **14**, a new allocolchicine derivative.

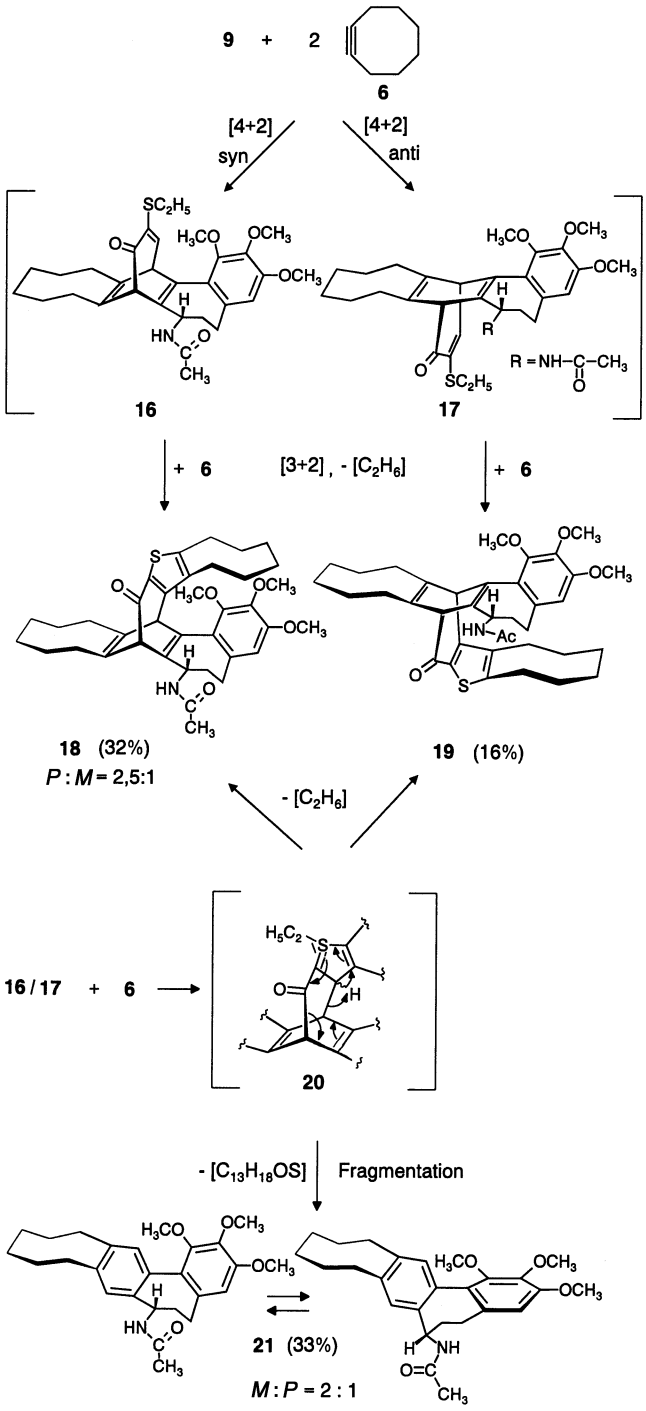
The cycloaddition behavior of cyclooctyne (**6**) was similar to that observed for **4** but proceeded with lower π-facial diastereoselectivity although *syn* approach was again predominant. Heating a mixture of enantiomerically pure 10-ethylthiocolchicide (**9**) with a tenfold excess of **6** in dry xylene at ca. 140°C for 13 h led to a mixture of three compounds in more than 80% total yield. From this mixture three compounds, the polycyclic species **18** and **19** and the allocolchicine derivative **21** could be separated by careful column chromatography in a 2:1:2 ratio.

The structures of the reaction products could be unambiguously elucidated by mass-, ^{1}H- and ^{13}C-NMR-spectral analyses (see Experimental Section), that of **19** additionally by X-ray crystal structure determination (Figure 2).

The identical parent ion peaks of **18** and **19** in the MS ($m/z = 615$ [M^+]) together with the ^{1}H- and ^{13}C-NMR data indicated, that these products must be stereoisomers. However, the spectroscopic data did not allow an unequivocal assignment of *syn* or *anti* configuration. Therefore, the less polar substance of the two, − eluted as second fraction after chromatographic separation − which yielded suitable crystals from $CHCl_3$/*n*-hexane (1:1), was submitted to X-ray analysis. This showed it to be the *anti* isomer **19**. Thus, the other stereoisomer **18** can be assigned the *syn* cycloadduct.

Both thiophene derivatives **18** and **19** are thermally stable compounds, even when they are heated for 24 h at 140°C in xylene (TLC protocol). Thus, the formation of the allocolchicinoid **21** by fragmentation of **18** or **19** can be ex-

Scheme 4



cluded. Like allocolchicinoid **14** compound **18** exists as a mixture of two atropo-diastereomeric conformations with an (*M*)/(*P*) = 1:2.5 ratio as indicated from ^{1}H-NMR data. In a similar fashion the biaryl axis of **21** is configuratively unstable; rotation around the A−C pivot bond gives rise to interconverting helicene-like distorted atropo-diastererомers with an (*M*)-**21**/(*P*)-**21** = 2:1 ratio. The ^{1}H-NMR behavior of **21** is similar to that of **14** and the ratio of the two atropodiastereomers in solution is solvent-dependent. Whereas in $CDCl_3$ solution the ratio (*M*)-**21**/(*P*)-**21** = 2:1, as indicated by ^{1}H-NMR data, in CD_3OD a total conformational conversion of (*P*)-**21** to (*M*)-**21** is observed, so that only the signals of (*M*)-**21** appear in the ^{1}H-NMR spectrum. This becomes also evident from optical rotation data. Again, the optical rotation distinctly shifted to a more negative value on switching solvents from $CHCl_3$ to CH_3OH. Although other reaction routes cannot be excluded, we suppose it to be reasonable that − analogously to the reaction mode of **4** − the [4+2] cycloaddition of **6** towards ethylthiocolchicide **9** is probably the first step of the reaction, in which the *syn* approach is predominating thus yielding the Diels-Alder adduct **16** as the main product besides **17**. The subsequent [3+2] cycloaddition of **6** toward the thienol ether moiety of both **16** and **17** furnishes intermediates like **20** which obviously reacts in two directions similar to the reaction course proposed for the formation of **13** and **14** (Scheme 3). Formal elimination of $[C_2H_6]$ leads to the thiophene derivatives **18** and **19**, respectively. The formation of the cyclooctene-annulated allocolchicine **21** can be understood to occur through heat-induced fragmentation of the intermediate **20**.

The colchicine analogs **10**, **14**, and **21** have been tested for the inhibition of microtubule assembly in vitro (see Experimental Section). Interestingly, the allocolchicine derivative **14** ($IC_{50} = 2.9 \cdot 10^{-5}$ M) was found to be only slightly less active than (−)-colchicine (**5**) ($IC_{50} = 9.3 \cdot 10^{-6}$ M). The compounds **10** and **21** can be considered to be inactive in relation to the activity of (−)-colchicine (**5**); the percentage of inhibition (**10**: 35% of inhibition, 10 mg/ml; **21**: 40% of inhibition, 10 mg/ml) only indicates, that these compounds belong to the colchicine family.

In conclusion we have shown, that Diels-Alder cycloadditions of (−)-(*M*,7*S*)-colchicine (**5**) with benzyne (**3**), DMAD (**4**), and cyclooctyne (**6**) proceeded with high positional and π-facial selectivity yielding novel cycloadducts with an intriguing homobarrelenone moiety.

In contrast to these findings the corresponding reactions of (−)-(*M*,7*S*)-10-ethylthiocolchicide (**9**) are considerably more complex. The new and unprecedented reaction routes observed verified that **9** is a substrate of ambident character with two functional sites. Thus, two independent reaction modes were observed, a [4+2] cycloaddition at the 8,12-positions of the alkaloid **9**, again preferentially from the diene face *syn* to the allylic substituent at the stereogenic center C-7, followed by a proposed [3+2] cycloaddition of the thioenol ether moiety of **9**. In the case of DMAD (**4**) and cyclooctyne (**6**) as starting materials the latter probably leads to intermediates like **15b** and **20**. These suffer fragmentation in two directions, both of them including the generation of a new aromatic 6π-system. This could be considered to be the driving force for the formation of the thiophene derivatives **13** and **18**, **19** on the one side and the biphenylic species **14** and **21** on the other side, axially chiral biphenyls characterized by a solvent-dependent conformational equilibrium of two atropo-diastereomeric forms.

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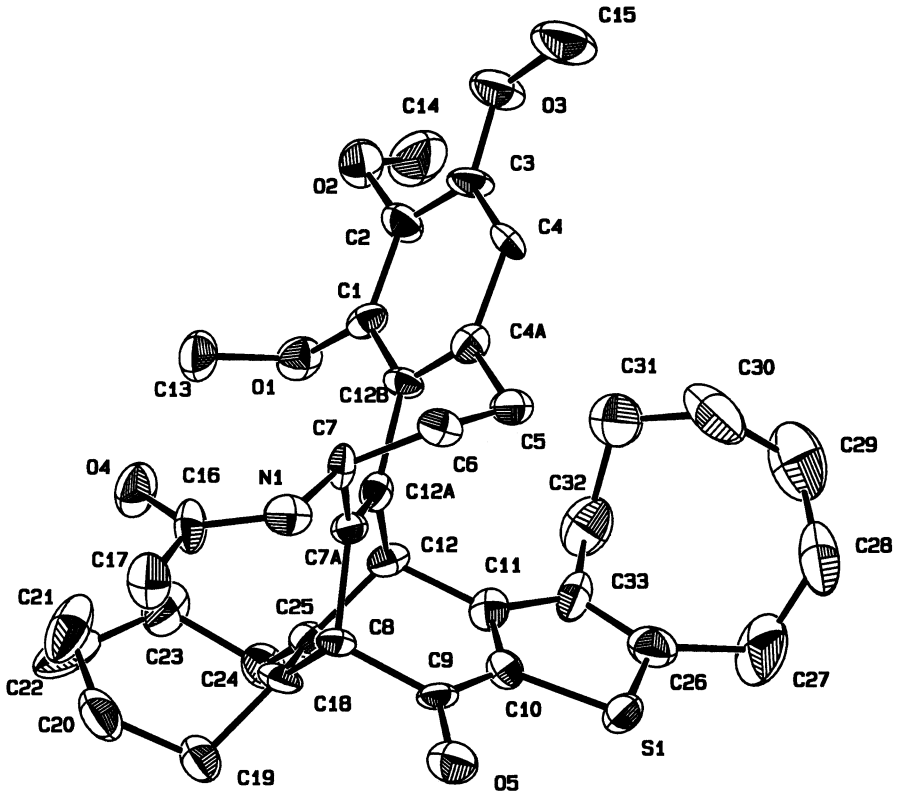
Figure 2. ZORTEP plot of the structure of cycloadduct **19** in the crystal[4]; thermal ellipsoids at the 40% probability level, the numbering of the atoms differs from that given in the experimental section



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Experimental Section

General Remarks: See ref.[8].

8,12-Dihydro-8α,12α-o-benzenocolchicine (**1**): a) To a magnetically stirred solution of 400 mg (1.0 mmol) of **5** and 443 mg (1.0 mmol) of lead tetraacetate in 10 ml of dry benzene was added a solution of 134 mg (1.0 mmol) of 1-aminobenzotriazole in 3 ml of dry benzene during 15 min. After filtration, the solution was concentrated in vacuo and the residue was purified by column chromatography (silica gel, column 20 × 3 cm, CH_2Cl_2/CH_3OH, 9.5:0.5). Fraction 1 contained **1**, which was recrystallized from CH_2Cl_2/*n*-hexane (1:2); fraction 2 contained 310 mg (77%) of the starting material **5**.

b) A solution of 0.4 ml (3.0 mmol) of isopentyl nitrite in 10 ml of dioxane and a solution of 411 mg (3.0 mmol) of anthranilic acid in 10 ml of dioxane were added concurrently, during 40 min, to a solution of 400 mg (1.0 mmol) of **5** in 10 ml of boiling dioxane. The solution was refluxed for 30 min and after cooling to room temp. concentrated in vacuo. The residue was purified by column chromatography (silica gel, column 20 × 3 cm, CH_2Cl_2/CH_3OH, 9.5:0.5); fraction 1 contained **1**, fraction 2 contained 108 mg (27%) of starting material **5**.

Yield: a) 96 mg (20%); b) 189 mg (40%), light yellow crystals, m. p. 232°C (dec.). − IR (KBr): $\tilde{\nu}$ = 3252 cm^{-1}(ν_{N-H}), 1689 , 1653 ($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 273 nm (4.161), 211 (4.578). − $[\alpha]_D^{20}$ = −286 (*c* = 0.44, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.84 (m, 1 H, 6-H_a), 1.88 (m, 1 H, 5-H_a), 2.03 (s, 3 H, COCH_3), 2.25 (m, 1 H, 5-H_b), 2.31 (m, 1 H, 6-H_b), 3.49 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 4.50 (m, 1 H, 7-H), 4.53 (d, ^3J = 9.2 Hz, 1 H, 12-H), 4.92 (s, 1 H, 8-H), 5.93 (d, ^3J = 7.5 Hz, 1 H, N*H*), 6.52 (s, 1 H, 4-H), 6.61 (d, ^3J = 9.2 Hz, 1 H, 11-H), 7.11 (m, 2 H, aromatic-H), 7.15 (m, 1 H, aromatic H), 7.38 (m, 2 H, aromatic-H). − ^{13}C NMR ($CDCl_3$): δ = 23.12 (CH_3, CO*C*H_3), 31.57 (CH_2, C-5), 39.60 (CH_2, C-6), 48.18 (CH, C-12), 49.15 (CH, C-7), 54.73 (O*C*H_3), 56.13 (O*C*H_3), 61.23 (O*C*H_3), 61.72 (CH, C-8), 61.95 (O*C*H_3), 108.66 (CH, C-4), 122.88 (CH, C-11) 124.57 (CH, aromatic-C), 124.80 (C-12b), 125.63 (CH, aromatic-C), 126.72 (2 × CH, aromatic-C), 135.57 (C-4a), 137.08 (C-12a/C-13), 141.40 (C-2), 142.14 (C-7a*), 145.14 (C-14*), 145.75(C-10), 151.99 (C-1), 153.17 (C-3), 169.97 (*C*OCH$_3$), 186.72 (C-9), *assignments not confirmed. − MS; *m/z* (%): 475 (100) [M^+], 457 (27), 432 (33), 416 (57). − $C_{28}H_{29}NO_6$: calcd. 475.1995, found 475.2002 (MS).

Crystal-Structure Determination of **1**: A yellow, transparent single crystal (ca. 0.3 × 0.25 × 0.2 mm), obtained by recrystallisation from CH_2Cl_2/*n*-hexane was investigated on a four-circle diffractometer (Enraf-Nonius CAD 4) by using graphite-monochromated Mo-K_α radiation at 24°C, ω-scan mode; empirical formula: $C_{28}H_{29}NO_6$, molecular mass 475.52, absorption coefficient μ = 0.09 mm^{-1}. The lattice constants of the hexagonal unit cell, space group $P3_2$ (*Z* = 6, $d_{calcd.}$ = 1.275 mg mm^{-3}) were refined with the

diffraction angles of 25 reflections in the 2θ range of 16−24° to furnish $a = 1355.4(2)$, $c = 1168.2(2)$ pm. For the refinement, 3449 independent reflections out of 4761 measured reflections ($R_{int} = 0.0466$) were used in the 2θ range of 4−50° ($0 \le h \le 16$, $-16 \le k \le 13$, $-13 \le l \le 13$). The structure was solved by direct methods[4a] and subjected to full-matrix least-square refinement based on F^2 values[4a], with anisotropic displacement factors for all heavier atoms (data/parameter = 11:1). The hydrogen atoms were included at idealized positions with fixed temperature factors using a riding model, only the hydrogen atom at N was refined free. Final reliability factors: $R_1 = 0.0544$ for 2225 reflections with $I > 2\sigma(I)$, $wR_2 = 0.1008$ {$w = 1/[\sigma^2(F_o)^2 + (0.0390P)^2]$ with $P = [F_o^2 + 2F_c^2]/3$} for all data. The largest difference peak is 0.129e/Å^3.

Dimethyl 8,12-Dihydro-8α,12α-ethenocolchicine-13,14-dicarboxylate (**2**): A solution of 400 mg (1.0 mmol) of **5** and 222 mg (1.5 mmol) of **4** in 5 ml of dry xylene was refluxed for 24 h under argon. The solvent was evaporated in vacuo and the residue was purified by column chromatography (silica gel, column 40 × 3 cm, CH_2Cl_2/CH_3OH, 9:1). Fraction 1 contained the excess of **4**, fraction 2 contained an unidentified product (1.5% yield). From fraction 3 the main product **2** was isolated, which could be recrystallized from $(Et)_2O/CH_2Cl_2$ (1:1). − Yield 355 mg (65%), colorless crystals, m. p. 136°C. − IR (KBr): $\tilde{\nu}$ = 3376 cm^{-1}(ν_{N-H}), 1720, 1686, 1658 ($\nu_{C=O}$). − UV (CH_2Cl_2): λ_{max} (lg ε) = 357 nm (2.93), 287 (3.88). − $[\alpha]_D^{20} = -206$ ($c = 0.56$, CH_2Cl_2). − ^{1}H NMR ($CDCl_3$): δ = 1.87 (m, 1 H, 6-H$_a$), 1.92 (s, 3 H, COC*H*$_3$), 2.16 (m, 1 H, 5-H$_a$), 2.25 (m, 1 H, 6-H$_b$), 2.33 (m, 1 H, 5-H$_b$), 3.47 (s, 3 H, OC*H*$_3$), 3.71 (s, 6 H, 2 × OC*H*$_3$), 3.77 (s, 3 H, OC*H*$_3$), 3.80 (s, 3 H, OC*H*$_3$), 3.83 (s, 3 H, OC*H*$_3$), 4.30 (d, ^3J = 9.1 Hz, 1 H, 12-H), 4.41 (m, 1 H, 7-H), 4.86 (s, 1 H, 8-H), 6.06 (d, ^3J = 7.8 Hz, 1 H, N*H*), 6.40 (d, ^3J = 9.1 Hz, 1 H, 11-H), 6.49 (s, 1 H, 4-H). − ^{13}C NMR ($CDCl_3$): δ = 23.03 (CH_3, CO*C*H$_3$), 31.63 (CH_2, C-5), 39.55 (CH_2, C-6), 45.43 (CH, C-12), 48.91 (CH, C-7), 52.61 (CH_3, COO*C*H$_3$), 52.75 (CH_3, COO*C*H$_3$), 54.73 (O*C*H$_3$), 56.11 (O*C*H$_3$), 58.66 (CH, C-8), 61.18 (O*C*H$_3$), 61.78 (O*C*H$_3$), 108.40 (CH, C-4), 120.14 (CH, C-11), 123.83 (C-12b), 135.58 (C-4a), 136.48 (C-12a*), 136.55 (C-7a*), 140.71 (C-13), 141.23 (C-2), 145.54 (C-10) 149.28 (C-14), 151.91 (C-1), 153.46 (C-3), 165.70 (*C*OOCH$_3$), 166.41 (*C*OOCH$_3$), 170.09 (*C*OCH$_3$), 182.92 (C-9), *assignments not confirmed. − MS; *m/z* (%): 541 (21) [M$^+$], 482 (52), 450 (100). − $C_{28}H_{31}NO_{10}$: calcd. 541.1948, found 541.1930 (MS).

Reaction of **5** *with Cyclooctyne* (**6**): A solution of 500 mg (1.25 mmol) of **5** and 1.08 g (10 mmol) of **6** in 5 ml of dry xylene was refluxed under argon for 24 h. The solvent was evaporated in vacuo and the residue purified and separated by column chromatography (silica gel, column 20 × 3 cm, CH_2Cl_2/CH_3OH, 9.5:0.5 to 9:1). Fraction 1 contained **8**, while fraction 2 contained **7**. Evaporation of the solvent of both fractions afforded amorphous powders. **8** was crystallized from CH_2Cl_2/*n*-hexane (1:1), **7** could only be obtained as amorphous powder, not in crystalline form.

Fraction 1: 8,12-Dihydro-8ß,12ß-[1,2]cyclooct[1]eno-colchicine (**8**): Yield 107 mg (17%), colorless crystals, m. p. 239°C. − IR (KBr): $\tilde{\nu}$ = 3266 cm^{-1}(ν_{N-H}), 1687, 1638($\nu_{C=O}$). − UV (CH_2Cl_2): λ_{max} (lg ε) = 330 nm (2.93), 259 (3.74). − $[\alpha]_D^{20} = -127$ (c = 0.25, CH_2Cl_2). − ^{1}H NMR ([D$_6$]DMSO): δ = 1.31 (m, 4 H, cyclooctено-H), 1.30−1.70 (m, 4 H, cycloocteno-H), 1.80 (m, 1 H, 6-H$_a$), 1.84 (s, 3 H, COC*H*$_3$), 2.05−2.25 (m, 3 H), 2.32 (m, 2 H), 2.41 (m, 2 H), 3.31 (s, 3 H, OC*H*$_3$), 3.66 (s, 3 H, OC*H*$_3$), 3.75 (s, 3 H, OC*H*$_3$), 3.79 (s, 3 H, OC*H*$_3$), 3.93 (d, ^3J = 9.2 Hz, 1 H, 12-H), 4.07 (s, 1 H, 8-H), 4.15 (m, 1 H, 7-H), 6.37 (d, ^3J = 9.2 Hz, 1 H, 11-H), 6.73 (s, 1 H, 4-H), 8.16 (d, ^3J = 7.4 Hz, 1 H, N*H*). − ^{13}C NMR ([D$_6$]DMSO): δ = 22.23 (CO*C*H$_3$), 25.46 (CH_2, cyclooctene), 25.85 (CH_2, cyclooctene), 27.64 (CH_2, cyclooctene), 28.42 (CH_2, cyclooctene), 30.79 (CH_2, C-5), 38.05 (2xCH_2, cyclooctene), 40.00 (CH_2, C-6), 47.75 (CH, C-7*), 47.85 (CH, C-12*), 54.22 (O*C*H$_3$), 55.80 (O*C*H$_3$), 60.41 (O*C*H$_3$), 60.62 (O*C*H$_3$), 62.97 (CH, C-8), 108.50 (CH, C-4), 121.01 (CH, C-11), 123.83 (C-12b), 131.56 (C-4a), 135.28 (C-12a), 136.60 (C-13*), 140.35 (C-2*), 140.67 (C-7a*), 144.14 (C-14*) 146.03 (C-10), 150.37 (C-1), 152.29 (C-3), 168.52 (*C*OCH$_3$), 186.90 (C-9), *assignments not confirmed. − MS; *m/z* (%): 507 (45) [M$^+$], 448 (100). − $C_{30}H_{37}NO_6$: calcd. 507.2621, found 507.2629 (MS).

Fraction 2: 8,12-Dihydro-8α,12α-[1,2]cyclooct[1]eno-colchicine (**7**): Yield 487 mg (77%), colorless amorphous powder, m. p. 126°C (dec.). − IR (KBr): $\tilde{\nu}$ = 3340 cm^{-1}(ν_{N-H}), 1666($\nu_{C=O}$). − UV (CH_2Cl_2): λ_{max} (lg ε) = 330 nm (2.99), 276 (4.04). − $[\alpha]_D^{20} = -197$ (c = 0.18, CH_2Cl_2). − ^{1}H NMR ([D$_6$]DMSO): δ = 1.33 (br. s, 6 H, cyclooctено-H), 1.54 (m, 2 H, cycloocteno-H), 1.77 (s, 3 H, COC*H*$_3$) 1.90 (m, 1 H, 6-H$_a$), 2.07 (m, 2 H), 2.25−2.40 (m, 5 H), 3.36 (s, 3 H, OC*H*$_3$), 3.66 (d, ^3J = 9.0 Hz, 1 H, 12-H), 3.72 (s, 3 H, OC*H*$_3$), 3.77 (s, 3 H, OC*H*$_3$), 3.80 (s, 3 H, OC*H*$_3$), 4.16 (m, 1 H, 7-H), 4.34 (s, 1 H, 8-H), 6.52 (d, ^3J = 9.0 Hz, 1 H, 11-H), 6.72 (s, 1 H, 4-H), 8.10 (d, ^3J = 8.5 Hz, 1 H, N*H*). − ^{13}C NMR ($CDCl_3$): δ = 23.11 (CO*C*H$_3$), 26.23 (CH_2, cyclooctene), 26.41 (CH_2, cyclooctene), 28.23 (CH_2, cyclooctene), 29.38 (CH_2, cyclooctene*), 31.23 (CH_2, cyclooctene*), 31.59 (CH_2, cyclooctene*), 31.77 (CH_2, C-5*), 39.99 (CH_2, C-6), 49.10 (CH, C-7*), 50.38 (CH, C-12*) 54.79 (O*C*H$_3$), 56.06 (O*C*H$_3$), 61.16 (O*C*H$_3$), 61.83 (O*C*H$_3$), 64.25 (CH, C-8), 108.65 (CH, C-4), 123.16 (CH, C-11), 125.16 (C-12b), 132.65 (C-4a), 135.57 (C-12a), 136.29 (C-13*), 141.22 (C-2), 141.69 (C-7a), 145.68 (C-14*) 146.13 (C-10), 151.83 (C-1), 152.76 (C-3), 170.03 (*C*OCH$_3$), 187.00 (C-9), *assignments not confirmed. − MS; *m/z* (%): 507 (23) [M$^+$], 448 (100). − $C_{30}H_{37}NO_6$: calcd. 507.2621, found 507.2611 (MS).

11-[2-(Ethylthio)phenyl]-10-demethoxycolchicine (**10**): A solution of 0.1 ml (0.75 mmol) of isopentyl nitrite in 7 ml of dioxane and a solution of 103 mg (0.75 mmol) anthranilic acid in 7 ml of dioxane were added concurrently, during 20 min, to a solution of 408 mg (0.95 mmol) of **9** in 10 ml of boiling dioxane. The mixture was refluxed for 30 min and after cooling to room temp. concentrated in acuo. The residue was purified by column chromatography (silica gel, column 20 × 3 cm, CH_2Cl_2/CH_3OH, 9.7:0.3). Fraction 1 contained 208 mg (51%) of the starting material **9**, fraction 2 contained compound **10**: Yield 144 mg (30%), yellowish amorphous powder, m. p. 112−115°C. − IR (KBr): $\tilde{\nu}$ = 3281 cm^{-1}(ν_{N-H}), 1779, 1732, 1653($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 372 nm (3.902), 335 (3.984), 238 (4.413), 210 (4.686). − $[\alpha]_D^{20} = -205$ (c = 0.4, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.20 (t, ^3J = 7.3 Hz, 3 H, SCH_2CH_3), 1.94 (m, 1 H, 6-H$_a$), 1.98 (s, 3 H, COC*H*$_3$), 2.25 (m, 1 H, 6-H$_b$), 2.54 (m, 2 H, 5-H), 2.83 (m, 2 H, SC*H*$_2$), 3.69 (s, 3 H, OC*H*$_3$), 3.85 (s, 6 H, 2 × OC*H*$_3$), 4.64 (m, 1 H, 7-H), 6.48 (s, 1 H, 4-H), 7.08 (dd, ^4J = 2.7 Hz, ^4J = 1.6 Hz, 1 H, 10-H), 7.19 (d, ^4J = 1.6 Hz, 1 H, 8-H), 7.19 (m, 2 H, phenyl-H), 7.27−7.35 (m, 2 H, phenyl-H), 7.47 (d, ^{4}J = 2.7 Hz, 1 H, 12-H), 8.13 (d, ^3J = 6.8 Hz, 1 H, N*H*). − ^{13}C NMR ($CDCl_3$): δ = 14.07 (CH_3, SCH_2CH_3), 22.93 (CH_3, CO*C*H$_3$), 27.91 (S-*C*H$_2$), 29.98 (CH_2, C-5), 35.96 (CH_2, C-6), 52.47 (CH, C-7), 56.18 (O*C*H$_3$), 61.45 (O*C*H$_3$), 61.85 (O*C*H$_3$), 107.26 (CH, C-4), 125.68 (C-12b), 125.93 (CH, phenyl-C), 128.80 (CH, phenyl-C), 128.92 (CH, phenyl-C), 129.04 (CH, phenyl-C), 134.25 (C-1′*), 134.65 (C-4a*), 135.27 (CH, C-12), 140.72 (CH, C-8), 140.89 (CH, C-10), 141.61(C-2), 142.54 (C-2′*), 143.41 (C-12a*), 149.80 (C-11*), 151.52 (C-1), 152.30(C-7a*), 153.84 (C-3*), 170.07 (*C*OCH$_3$), 186.54 (C-9), *assignments not confirmed. − MS; *m/z* (%): 505 (80) [M$^+$], 477 (10), 434 (100). − $C_{29}H_{31}NO_5S$: calcd. 505.1923, found 505.1928 (MS).

Reaction of **9** *with Dimethyl Acetylenedicarboxylate* (**4**): A solution of 430 mg (1.0 mmol) of **9** and 0.5 ml (4.1 mmol) of **4** in 10 ml of dry toluene was heated in a pressure bottle at 125−130°C for 40 h. After cooling to room temp., the solvent was evaporated in vacuo. TLC control exhibited that a complex mixture of products was formed with two main products, which could be separated after repeated column chromatography (silica gel, column 20 × 3 cm, ethyl acetate).

Tetramethyl [7S-(7α,8α,13α)]-7-(Acetylamino)-5,6,7,8,9,13-hexahydro-1,2,3-trimethoxy-9-oxo-8,13-ethenobenzo[6′,7′]cyclohepta[1′,2′:4,5]cycloheptа[1,2-b]thiophene-11,12,14,15-tetracarboxylate (**13**): R_f = 0.26, Yield 80 mg (12%), light yellow crystals, m. p. 228−230°C (ethyl acetate/*n*-hexane, 1:1). − IR (KBr): $\tilde{\nu}$ = 3396 cm^{-1}(ν_{N-H}), 1720, 1699, 1678, 1652 ($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 296 nm (4.147), 244 (4.397), 214 (4.642). − $[\alpha]_D^{20}$ = −66 (*c* = 0.36, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 1.94 (m, 1 H, 6-H_a), 2.01 (s, 3 H, COC*H*$_3$), 2.28 (m, 2 H, 5-H/6-H_b), 2.43 (m, 1 H, 5-H), 3.17 (s, 3 H, OC*H*$_3$), 3.78 (s, 3 H, OC*H*$_3$), 3.79 (s, 3 H, OC*H*$_3$), 3.84 (s, 3 H, OC*H*$_3$), 3.85 (s, 6 H, 2 × OC*H*$_3$), 3.88 (s, 3 H, OC*H*$_3$), 4.43 (m, 1 H, 7-H), 4.96 (s, 1 H, 8-H), 5.16 (s, 1 H, 13-H), 5.93 (d, ^3J = 7.3 Hz, 1 H, N*H*), 6.52 (s, 1 H, 4-H). − ^{13}C NMR ($CDCl_3$): δ = 22.94 (CH_3, CO*C*H$_3$), 31.36 (CH_2, C-5), 38.99 (CH_2, C-6), 47.68 (CH, C-13), 49.16 (CH, C-7), 52.42 (CH_3, CO-O*C*H$_3$), 52.72 (CH_3, COO*C*H$_3$), 52.84 (CH_3, COO*C*H$_3$), 53.05 (CH_3, COO*C*H$_3$), 56.21 (O*C*H$_3$), 56.57 (CH, C-8), 60.45 (O*C*H$_3$), 61.03 (O*C*H$_3$), 108.61 (CH, C-4), 124.25 (C-13b), 134.07 (C-12), 135.22(C-4a), 136.02 (C-13a*), 136.10 (C-7a*), 137.22 (C-9a/C-11), 140.01 (C-14), 141.47 (C-2), 150.44 (C-15), 151.43 (C-1), 152.39 (C-12a*), 153.54 (C-3*), 161.18 (*C*OOCH$_3$), 163.10 (*C*OOCH$_3$), 165.16 (*C*OOCH$_3$), 166.44 (*C*OOCH$_3$), 170.25 (*C*OCH$_3$), 180.09 (C-9), * assignments not confirmed. − MS; *m/z* (%): 683 (46) [M^+], 640 (31), 624 (44), 592 (100). − $C_{33}H_{33}NO_{13}S$: calcd. 683.1673, found 683.1715 (MS).

Dimethyl (S)-5-(Acetylamino)-6,7-dihydro-9,10,11-trimethoxy-5H-dibenzo[a,c]cycloheptene-2,3-dicarboxylate (**14**): R_f = 0.20, Yield 59 mg (13%), colorless amorphous powder, m. p. 100−102°C. − IR (KBr): $\tilde{\nu}$ = 3287 cm^{-1}(ν_{N-H}), 1726, 1661 ($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 299 nm (4.101), 282 (4.127), 243 (4.305), 211 (4.647). − $[\alpha]_D^{20}$ = −108 (*c* = 0.34, $CHCl_3$), $[\alpha]_D^{20}$ = −113 (*c* = 0.27, CH_3OH). − ^{1}H NMR ($CDCl_3$), (*M*) conformer: δ = 1.79 (m, 1 H, 6-H_a), 2.05 (s, 3 H, COC*H*$_3$), 2.18 (m, 1 H, 7-H), 2.44 (m, 2 H, 7-H/6-H_b), 3.57 (s, 3 H, OC*H*$_3$), 3.905 (s, 3 H, OC*H*$_3$), 3.907 (s, 3 H, OC*H*$_3$), 3.927 (s, 3 H, OC*H*$_3$), 3.928 (s, 3 H, OC*H*$_3$), 4.85 (m, 1 H, 5-H), 6.10 (d, ^3J = 7.6 Hz, 1 H, N*H*), 6.57 (s, 1 H, 8-H), 7.64 (s, 1 H, 4-H), 7.86 (s, 1 H, 11-H). − ^{13}C NMR ($CDCl_3$), (*M*) conformer: δ = 23.24 (CH_3, CO*C*H$_3$), 30.19 (CH_2, C-7), 39.26 (CH_2, C-6), 49.15 (CH, C-5), 52.58 (CH_3, COO*C*H$_3$), 52.65 (CH_3, COO*C*H$_3$), 56.06 (O*C*H$_3$), 61.23 (O*C*H$_3$), 61.35 (O*C*H$_3$), 107.74 (CH, C-8), 123.07 (C-11a), 123.37 (CH, C-4), 130.00 (C-3*), 130.34 (C-2*), 130.92 (CH, C-1), 134.66 (C-7a), 137.79 (C-11b), 141.37 (C-10), 142.79 (C-4a), 151.20 (C-11), 153.54 (C-9), 168.01 (*C*O), 168.53 (*C*O), 169.39 (*C*O), *assignments not confirmed. − MS; *m/z* (%): 457 (100) [M^+], 426 (36), 398 (59). − $C_{24}H_{27}NO_8$: calcd. 457.1737, found 457.1721 (MS).

The ^{1}H- and ^{13}C-NMR data of the (*P*) conformer could not be obtained completely.

Reaction of **9** *with Cyclooctyne* (**6**): A solution of 430 mg (1.0 mmol) of **9** and 1.08 g (10 mmol) of **6** in 5 ml of dry xylene was refluxed under argon for 13 h. The solvent was evaporated in vacuo and the residue was purified and separated by column chromatography (silica gel, 20 × 3 cm, CH_2Cl_2/MeOH, 9.8:0.2). Fraction 1 contained **21**, fraction 2 contained **19** and fraction 3 contained **18**. Evaporation of the solvent of all fractions afforded crystalline residues, which were recrystallized from $CHCl_3$/*n*-hexane (1:1). **21** and **18** are mixtures of two conformers − (*M*)/(*P*) ratio = 2:1 (**21**) and 1:2.5 (**18**).

[7S-(7α,8α,17α)]-N-(5,6,7,8,9,11,12,13,14,15,16,17,20,21,22,23,24,25-Octadecahydro-1,2,3-trimethoxy-9-oxo-8,17[1′,2′]-endocyclooctabenzo[6′,7′]cyclohepta[1′,2′:4,5]cyclohepta[1,2-b]cyclooctа[d]thiophen-7-yl)acetamide (**18**): Yield 200 mg (32%), colorless amorphous powder, m. p. 137°C. − IR (KBr): $\tilde{\nu}$ = 3327 cm^{-1}(ν_{N-H}), 1650 cm^{-1} ($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 337 nm (3.834), 296 (4.058), 268 (4.157), 208 (4.593). − $[\alpha]_D^{20}$ = −7 (*c* = 0.3, $CHCl_3$). − ^{1}H NMR ($CDCl_3$), (*P*) conformer: δ = 0.56 (m, 1 H, cyclooctene-H), 0.70 (m, 1 H, cyclooctene-H), 1.11 (m, 1 H, cyclooctene-H), 1.22 (m, 4 H, cyclooctene-H), 1.33 (m, 5 H, cyclooctene-H), 1.53 (m, 2 H, cyclooctene-H), 1.60 (s, 3 H, COC*H*$_3$), 1.67 (m, 2 H, cyclooctene-H) 2.01 (m, 2 H, 6-/5-H), 2.13 (m, 1 H, 5-H), 2.32−2.59 (m, 7 H, 6-/cyclooctene-H), 2.71 (m, 1 H, cyclooctene-H), 2.78 (m, 1 H, cyclooctene-H), 3.77 (s, 3 H, OC*H*$_3$), 3.85 (s, 3 H, OC*H*$_3$), 3.87 (s, 3 H, OC*H*$_3$), 4.21 (s, 1 H, 8-H), 4.50 (s, 1 H, 17-H), 4.66 (m, 1 H, 7-H), 5.21 (d, ^3J = 7.8 Hz, 1 H, N*H*), 6.47 (s, 1 H, 4-H). − (*M*) conformer: δ = 0.56−2.89 (28 H, 5-/6-/cyclooctene-H), 2.00 (s, 3 H, COC*H*$_3$), 3.07 (s, 3 H, OC*H*$_3$), 3.86 (s, 3 H, OC*H*$_3$), 3.91 (s, 3 H, OC*H*$_3$), 4.19 (s, 1 H, 8-H), 4.28 (s, 1 H, 17-H), 4.41 (m, 1 H, 7-H), 5.91 (d, ^3J = 7.6 Hz, 1 H, N*H*), 6.54 (s, 1 H, 4-H). − ^{13}C NMR ($CDCl_3$), (*P*) conformer: δ = 22.94 (CH_3, CO*C*H$_3$), 24.15 (CH_2, cyclooctene), 25.12 (CH_2, cyclooctene), 25.89 (CH_2, cyclooctene), 26.13 (CH_2, cyclooctene), 26.27 (CH_2, cyclooctene), 28.15 (CH_2, cyclooctene), 28.84 (CH_2, cyclooctene), 29.00 (CH_2, cyclooctene), 29.92 (CH_2, cyclooctene), 31.32 (CH_2, C-5), 31.46 (CH_2, cyclooctene), 31.54 (CH_2, cyclooctene), 32.45 (CH_2, cyclooctene), 41.44 (CH_2, C-6), 51.11 (CH, C-7*), 51.15 (CH, C-17*) 55.96 (O*C*H$_3$), 60.79 (O*C*H$_3$), 61.22 (O*C*H$_3$), 69.75 (CH, C-8), 107.90 (CH, C-4), 124.35 (CH, C-17b), 128.61 (C-16a), 134.10 (C-4a), 136.26 (C-17a*), 137.16 (C-9a*), 140.79 (C-18*), 140.98 (C-7a*), 141.60 (C-2*) 142.55 (C-10a*), 147.38 (C-19*), 151.04 (C-1), 152.82 (C-3), 154.33 (C-16b), 167.91 (*C*OCH$_3$), 186.56(C-9). − (*M*) conformer: δ = 23.15 (CH_3, CO*C*H$_3$), 24.49−31.97 (12 × CH_2, cyclooctene), 30.91 (CH_2, C-5), 39.25 (CH_2, C-6), 49.27 (CH, C-7), 52.95 (CH, C-17) 56.06 (O*C*H$_3$), 60.68 (O*C*H$_3$), 61.22 (O*C*H$_3$), 62.67 (CH, C-8), 108.41 (CH, C-4), 125.91 (CH, C-17b), 128.18 (C-16a), 135.29 (C-4a), 137.68 (C-17a*), 138.29 (C-9a*), 140.59 (C-18*), 140.79 (C-7a*), 141.29 (C-2*) 145.24 (C-10a*), 146.54 (C-19*), 152.01 (C-1), 152.51 (C-3), 155.57 (C-16b), 169.95 (*C*OCH$_3$), 185.66(C-9), *assignments not confirmed. − MS; *m/z* (%): 615 (64) [M^+], 572 (41), 556 (100). − $C_{37}H_{45}NO_5S$: calcd. 615.3018, found 615.3020 (MS).

[7S-(7α,8β,17β)]-N-(5,6,7,8,9,11,12,13,14,15,16,17,20,21,22,23,24,25-Octadecahydro-1,2,3-trimethoxy-9-oxo-8,17[1′,2′]-exocyclooctabenzo[6′,7′]cyclohepta[1′,2′:4,5]cyclohepta[1,2-b]cyclooctа[d]thiophen-7-yl)acetamide (**19**): Yield 101 mg (16%), colorless crystalls, m. p. 126°C. − IR (KBr): $\tilde{\nu}$ = 3343 cm^{-1}(ν_{N-H}), 1662 cm^{-1}, 1632 cm^{-1} ($\nu_{C=O}$). − UV (MeOH): λ_{max} (lg ε) = 344 nm (3.608), 315 (3.851), 265 (4.141), 210 (4.469). − $[\alpha]_D^{20}$ = −93 (*c* = 0.25, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 0.53 (m, 1 H, cyclooctene-H), 0.63 (m, 1 H, cyclooctene-H), 1.04 (m, 1 H, cyclooctene-H), 1.23 (m, 4 H, cyclooctene-H), 1.33 (m, 5 H, cyclooctene-H), 1.53 (m, 2 H, cyclooctene-H), 1.71 (m, 2 H, cyclooctene-H) 1.80 (m, 2 H, 6-/5-H), 2.01 (s, 3 H, COC*H*$_3$), 2.08 (m, 1 H, 5-H), 2.24 (m, 2 H, 6-H/cyclooctene-H), 2.37−2.54 (m, 5 H, cyclooctene-H), 2.67 (m, 1 H, cyclooctene-H), 2.74 (m, 1 H, cyclooctene-H), 3.74 (s, 3 H, OC*H*$_3$), 3.78 (s, 3 H, OC*H*$_3$), 3.85 (s, 3 H, OC*H*$_3$), 4.13 (s, 1 H, 8-H), 4.49 (s, 1 H, 17-H), 4.62 (m, 1 H, 7-H), 5.98 (m, 1 H, N*H*), 6.38 (s, 1 H, 4-H). − ^{13}C NMR ($CDCl_3$): δ = 23.20

(CH_3, $COCH_3$), 24.13 (CH_2, cyclooctene), 25.13 (CH_2, cyclooctene), 25.89 (CH_2, cyclooctene), 26.06 (CH_2, cyclooctene), 26.32 (CH_2, cyclooctene), 28.19 (CH_2, cyclooctene), 28.76 (CH_2, cyclooctene), 28.95 (CH_2, cyclooctene), 29.86 (CH_2, cyclooctene), 31.00 (CH_2, C-5), 31.65 (CH_2, cyclooctene), 31.67 (CH_2, cyclooctene), 32.52 (CH_2, cyclooctene), 40.88 (CH_2, C-6), 48.53 (CH, C-7), 50.86 (CH, C-17) 56.05 (OCH_3), 61.23 (OCH_3), 61.51 (OCH_3), 61.93 (CH, C-8), 108.07 (CH, C-4), 123.85 (CH, C-17b), 128.30 (C-16a), 133.16 (C-4a), 135.42 (C-17a*), 137.13(C-9a*), 137.54 (C-18*), 141.06 (C-7a*), 141.11 (C-2*) 143.07 (C-10a*), 147.80 (C-19*), 151.24 (C-1), 152.70 (C-3), 155.07 (C-16b), 169.34 ($COCH_3$), 187.84 (C-9), *assignments not confirmed. – MS; *m/z* (%): 615 (89) [M^+], 572 (51), 556 (100). – $C_{37}H_{45}NO_5S$: calcd. 615.3018, found 615.3026 (MS).

Crystal-Structure Determination of **19**: A colorless, single crystal was investigated with a four-circle diffractometer (Enraf-Nonius CAD4) by using graphite-monochromated Mo-K_α-radiation at 22°C, ω-scan mode; empirical formula: $C_{38}H_{46}Cl_3NO_5S$, molecular mass 735.21, absorption coefficient $\mu = 0.23$ mm^{-1}. The lattice constants of the monoclinic unit cell, space group $P2_1$ ($Z = 2$, $d_{calcd.} = 1.168$ mg mm^{-3}) were refined with the diffraction angles of 24 reflections in the 2θ range of 40–46° to furnish $a = 1283.6(10)$, $b = 1043.8(10)$, $c = 1435.9(8)$ pm, $\beta = 92.85(7)°$. For the refinement, 3581 independent reflections out of 7150 measured reflections ($R_{int} = 0.111$) were used in the 2θ range of 4–50° ($-15 \le h \le 15$, $0 \le k \le 12$, $-17 \le l \le 17$). An absorption correction was not carried out. The structure was solved by direct methods[4a] and subjected to full-matrix least-square refinement based on F^2 values[4a], with anisotropic displacement factors for all heavier atoms (data/parameter = 9:1). The hydrogen atoms were included at idealized positions with fixed temperature factors using a riding model. The disorder of one $CHCl_3$ molecule in the unit cell could not be described. Therefore, the contribution of the solvent molecule to the total structure factor was calculated and incorporated in the refinement of the ordered part with help of the "bypass" procedure of van der Sluis and Spek.[4b] Flack's absolute structure parameter: 0.2(2). Final reliability factors: $R = 0.059$ for 1848 reflections with $I < 2\sigma(I)$, $wR_2 = 0.133$($w = 1/[\sigma^2(F_o)^2 + (0.0399P)^2]$, with $P = [F_o^2 + 2F_c^2]/3$) for all data.

(S)-N-(6,7,9,10,11,12,13,14-Octahydro-1,2,3-trimethoxy-5H-benzo[3′,4′]cyclohepta[1′,2′:4,5]benzo[1,2]cycloocten-7-yl)acetamide (**21**): Yield 140 mg (33%), colorless crystalls, m. p. 103–106°C. – IR (KBr): $\tilde{\nu} = 3273$ cm^{-1}(ν_{N-H}), 1653 cm^{-1} ($\nu_{C=O}$). – UV (MeOH): λ_{max} (lg ε) = 261 nm (4.252), 215 (4.581). – $[\alpha]_D^{20} = -35$ ($c = 0.375$, $CHCl_3$), $[\alpha]_D^{20} = -61$ ($c = 0.25$, CH_3OH). – ^{1}H NMR ($CDCl_3$), (*M*) conformer: δ = 1.35 (m, 4 H, cyclooctено-H), 1.66 (m, 4 H, cyclooctено-H) 1.75 (m, 1 H, 6-H_a), 2.04 (s, 3 H, $COCH_3$), 2.30 (m, 1 H, 6-H_b), 2.40 (m, 2 H, 5-H), 2.75 (m, 4 H, cyclooctено-H), 3.46 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 4.83 (m, 1 H, 7-H), 5.83 (d, $^3J = 8.7$ Hz, 1 H, N*H*) 6.54 (s, 1 H, 4-H) 6.92 (s, 1 H, 8 H) 7.23 (s, 1 H, 15-H). – (*P*) conformer: δ = 1.35 (m, 4 H, cyclooctено-H), 1.57 (s, 3 H, $COCH_3$), 1.66 (m, 4 H, cyclooctено-H) 2.15 (m, 1 H, 6-H_a), 2.40 (m, 1 H, 6-H_b), 2.51 (m, 2 H, 5-H), 2.75 (m, 4 H, cyclooctено-H), 3.55 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 5.09 (m, 1 H, 7-H), 5.26 (d, $^3J = 9.1$ Hz, 1 H, N*H*) 6.63 (s, 1 H, 4-H) 7.03 (s, 1 H, 8 H) 7.24 (s, 1 H, 15-H). – ^{13}C NMR ($CDCl_3$), (*M*) conformer: δ = 23.48 (CH_3, $COCH_3$), 25.77 (CH_2, cyclooctene), 26.04 (CH_2, cyclooctene), 30.73 (CH_2, C-5), 31.72 (CH_2, cyclooctene), 32.37 (CH_2, cyclooctene), 32.42 (CH_2, cyclooctene), 32.54 (CH_2, cyclooctene), 40.05 (CH_2, C-6), 48.81 (CH, C-7), 56.03 (OCH_3), 60.91 (OCH_3), 61.29 (OCH_3), 107.57 (CH, C-4), 122.42 (CH, C-8), 125.11 (C-15b), 130.70 (CH, C-15), 132.13 (C-4a), 134.75 (C-15a*), 136.48 (C-7a*), 139.37 (C-14a*), 140.16 (C-8a*), 140.61 (C-2*), 151.27 (C-1), 152.39 (C-3), 168.52 ($COCH_3$); (*P*) conformer: δ = 23.37 (CH_3, $COCH_3$), 25.79 (CH_2, cyclooctene), 26.04 (CH_2, cyclooctene), 30.95 (CH_2, C-5), 31.82 (CH_2, cyclooctene), 32.08 (CH_2, cyclooctene), 32.11 (CH_2, cyclooctene), 32.54 (CH_2, cyclooctene), 41.07 (CH_2, C-6), 53.12 (CH, C-7), 56.07 (OCH_3), 60.37 (OCH_3), 61.24 (OCH_3), 107.99 (CH, C-4), 125.94 (C-15b), 129.56 (CH, C-8), 131.61 (CH, C-15), 131.84 (C-4a), 136.15 (C-15a*), 136.68 (C-7a*), 140.25 (C-2*), 141.30 (C-14a*), 141.40 (C-8a*), 150.99 (C-1), 152.71 (C-3), 167.65 ($COCH_3$), *assignments not confirmed. – MS; *m/z* (%): 423 (65) [M^+], 364 (100). – $C_{26}H_{33}NO_4$: calcd. 423.2410, found 423.2415 (MS).

Tubulin Binding Assay: Calf brain tubulin was purified according to the method of Shelanski[9], by three cycles of assembly-disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 2 mM EGTA, and 1 mM GTP pH = 6.6 (the concentration of tubulin was about 2–3 mg/ml). Tubulin assembly was monitored and recorded continuously by turbidimetry at 400 nm in a UV spectrophotometer, equipped with a thermostated cell at 37°C[10]. We determined for drug **14** the IC_{50} value of its concentration which decreased by 50% the maximum assembly rate of tubulin without drug. The IC_{50} for compound **14** was compared to the IC_{50} of colchicine, measured the same day under the same conditions. For compounds with low affinity, the percentage of inhibition of tubulin assembly for a concentration of 10 mg/ml is only indicated.

☆ Dedicated to Professor *G. Heinisch*, Innsbruck, on the occasion of his 60th birthday.

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