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A [2 + 2 + 2]-Cycloaddition Approach toward 6-Oxa-allocolchicinoids with Apoptosis-Inducing Activity

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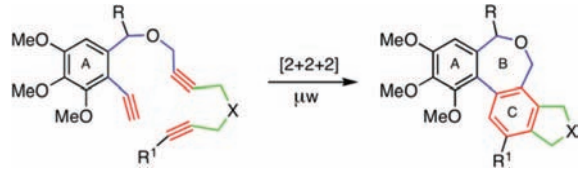
ABSTRACT



Following an A → ABC strategy, a new synthesis of 6-oxa-allocolchicinoids was developed exploiting a microwave-promoted Co- or Rh-catalyzed intramolecular [2 + 2 + 2]-cycloaddition (alkyne cyclotrimerization) as a key step. The approach opens a short and efficient access to a variety of novel compounds, some of which were found to exhibit significant and selective apoptosis-inducing activities against BJAB tumor cells.

Small molecules influencing the polymerization of tubulin (microtubule formation) are of great interest as potential new anticancer drugs.[1] One of the oldest known tubulin-binding agents is colchicine (**1**),[2] which induces microtubule depolymerization and is used as a drug against acute gout and familial Mediterranean fever.[3] While the high general toxicity of **1** prohibits its use in cancer therapy, its structure with the unique tricyclic scaffold represents an interesting lead for the development of new anticancer drugs targeting tubulin.[1]

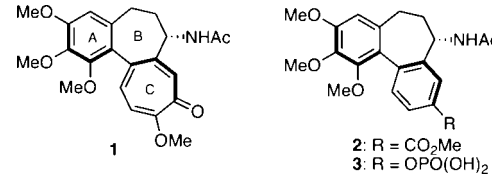


Figure 1. Colchicine (**1**), allocolchicine (**2**), and ZD6126 (**3**).

Allocolchicine (**2**) and related compounds, such as the colchinol derivative ZD6126 (**3**),[4] also exhibit promising biological activities[5] and are easier to handle due to the lack of the sensitive tropolone ether moiety. Thus, the development of synthetic approaches toward allocolchicine-related compounds represents a relevant task.[6]

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[‡] Humboldt University Berlin.

(1) Jordan, M. A.; Wilson, L. *Nature Rev. Cancer* **2004**, *4*, 253–265.
(2) Selected reviews on colchicine chemistry: (a) Capraro, H. G.; Brossi, A. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: Orlando, 1984; Vol. 23,Chapter 1. (b) Boyé, O.; Brossi, A. In *The Alkaloids*; Brossi, A., Cordell, G. A., Eds.; Academic Press: San Diego, 1992; Vol. 41, p 125. (c) Graening, T.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2004**, *43*, 3230–3256. For a structure, at 3.5 Å resolution, of tubulin in complex with colchicine, see: (d) Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198–202.
(3) (a) Le Hello, C. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 2000; Vol. 53, Chapter 5. (b) Wallace, S. L. *Arthritis Rheum.* **2006**, *2*, 389–395.
(4) Davis, P. D.; Dougherty, G. J.; Blakey, D. C.; Galbraith, S. M; Tozer, G. M.; Holder, A. L.; Naylor, M. A.; Nolan, J.; Stratford, M. R. L.; Chaplin, D. J.; Hill, S. A. *Cancer Res.* **2002**, *62*, 7247–7253.
(5) Boyé, O.; Brossi, A.; Yeh, H. J. C.; Hamel, E.; Wegrzynski, B.; Toome, V. *Can. J. Chem.* **1992**, *70*, 1237–1249.

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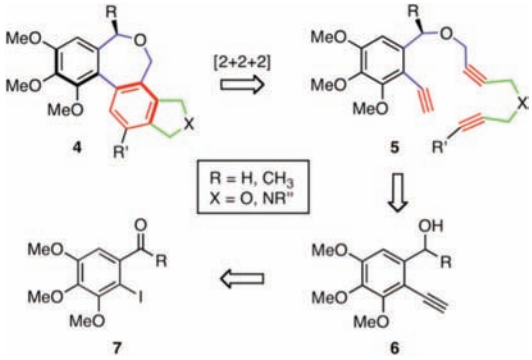
We have recently demonstrated in a total synthesis of **1**[7] that intramolecular cycloaddition strategies (following an A → ABC scheme) allow for the efficient construction of ring systems related to colchicine. We here disclose the application of a related concept to the synthesis of 6-oxa-allocolchicinoids of type **4**, some of which were found to possess pronounced apoptosis-inducing properties.

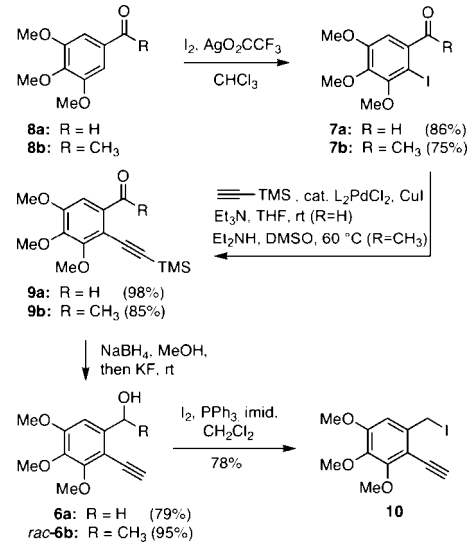
Scheme 1. Retrosynthetic Analysis



Our synthetic concept is outlined in Scheme 1. Exploiting a metal-catalyzed intramolecular [2 + 2 + 2]-cycloaddition (Reppe−Vollhardt alkyne cyclotrimerization)[8] as a key step, the target molecules would be derived from triynes of type **5**. These in turn could be assembled in a straightforward fashion (via **6**) starting from readily accessible precursors of type **7**. Besides requiring only a few linear steps, such a scheme would open access to a rather broad diversity of new allocolchicinoids (especially with respect to the ring C substitution pattern). Moreover, by means of a substituent R at ring B a conformational bias could be induced to also predetermine the biaryl twist.[9]

The preparation of the required phenylacetylene building blocks of type **6** started with the $Ag(CF_3CO_2)$-promoted iodination[10] of aldehyde **8a** and ketone **8b** (Scheme 2). The resulting products (**7a** and **7b**) were then used for the introduction of the first alkyne moiety through Sonogashira cross-coupling using $PdCl_2(PPh_3)_2$ and CuI as catalysts.[11] It is noteworthy that aldehyde **7a** reacted smoothly at room temperature in THF to give **9a** in high yield, whereas heating to 60 °C in DMSO was necessary to obtain full conversion in the case of **7b**. The carbonyl compounds **9a** and **9b** were then treated with $NaBH_4$ and KF in methanol (one-pot procedure) to afford the alcohols **6a** and *rac*-**6b**, respectively. In addition, treatment of alcohol **6a** with iodine in the presence of PPh_3 and imidazole[12] cleanly afforded the iodide **10** as an alternative building block.

Scheme 2. Preparation of Phenylacetylene Building Blocks



The next task was the preparation of cyclization precursors of type **5** (compare Scheme 1). First experiments in this direction were performed employing building block *rac*-**6b**, which was efficiently *O*-alkylated by the propargylic bromides **11** and **13** to afford the products *rac*-**12** (after THP cleavage) and *rac*-**14**, respectively, in good yield (Scheme 3). The nitrogen-containing triynes *rac*-**17** and *rac*-**18** were then prepared by reaction of *rac*-**12** with the sulfonamides **15** and **16** under Mitsunobu-type conditions.[13] Remarkably, all attempts to directly *O*-alkylate *rac*-**6b** with the tosyl-protected amine-analog of bromoether **13** only led to a complex mixture of "polymeric" products, which is why the synthesis of *rac*-**17** and *rac*-**18** had to be performed under nonbasic conditions via the three-step sequence described (Scheme 3).

The synthesis of the cyclization precursors **24**−**28** (formally derived from the primary benzylic alcohol **6a**) was efficiently achieved by NaH-promoted reaction of the ben-

(6) (a) Sawyer, J. S.; Macdonald, T. L. *Tetrahedron Lett.* **1988**, *29*, 4839–4842. (b) Vorogushin, A. V.; Predeus, A. V.; Wulff, W. D.; Hansen, H.-J. *J. Org. Chem.* **2003**, *68*, 5826–5831. (c) Leblanc, M.; Fagnou, K. *Org. Lett.* **2005**, *7*, 2849–2852. (d) Wu, T. R.; Chong, J. M. *Org. Lett.* **2006**, *8*, 15–18. (e) Besong, G.; Jarowicki, K.; Kocienski, P. J.; Sliwinski, E.; Boyle, F. T. *Org. Biomol. Chem.* **2006**, *4*, 2193–2207. (f) Seganish, W. M.; DeShong, P. *Org. Lett.* **2006**, *8*, 3951–3954. (g) Djurdjevic, S.; Green, J. R. *Org. Lett.* **2007**, *9*, 5505–5508. (h) Joncour, A.; Décor, A.; Liu, J.-M.; Tran Huu Dau, M.-E.; Baudoin, O. *Chem. Eur. J.* **2007**, *13*, 5450–5465. (i) Boyer, F.-D.; Hanna, I. *Org. Lett.* **2007**, *9*, 715–718. (j) Boyer, F.-D.; Hanna, I. *Eur. J. Org. Chem.* **2008**, 4938–4948.

(7) (a) Graening, T.; Friedrichsen, W.; Lex, J; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1524–1526. (b) Graening, T.; Bette, V.; Neudörfl, J.; Lex, J.; Schmalz, H.-G. *Org. Lett.* **2005**, *7*, 4317–4320.

(8) Selected reviews: Vollhardt, K. P. C. *Angew. Chem., Int. Ed.* **1984**, *23*, 539–559. (b) Kotha, S.; Brahmachary, E.; Lahiri, K. *Eur. J. Org. Chem.* **2005**, 4741–4767. (c) Chopade, P. R.; Louie, J. *Adv. Synth. Catal.* **2006**, *348*, 2307–2327. (d) Agenet, N.; Buisine, O.; Slowinski, F.; Gandon, V.; Aubert, C., Malacria, M. In *Organic Reactions*; Overman, L. E., Ed.; Wiley: New York, 2007; Vol. 68, pp 1−302.

(9) For the dependency of the biological acitivity of colchicine-related compounds on the configuration of the chiral (biaryl) axis, see: Berg, U.; Deinum, J.; Lincoln, P.; Kvassman, J. *Bioorg. Chem.* **1991**, *19*, 53–65.

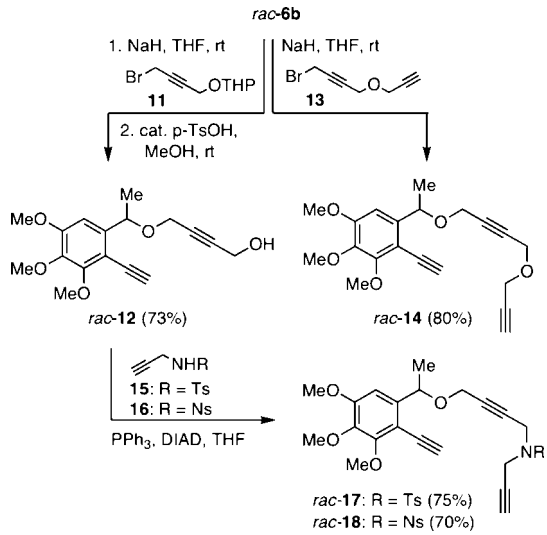
(10) Janssen, D. E.; Wilson, C. V. *Org. Synth.* **1956**, *36*, 46.

(11) For a related transformation, see: Lee, J. C.; Cha, J. K. *Tetrahedron* **2000**, *56*, 10175–10184.

(12) Lange, G. L.; Gottardo, C. *Synth. Commun.* **1990**, *20*, 1473–1479.

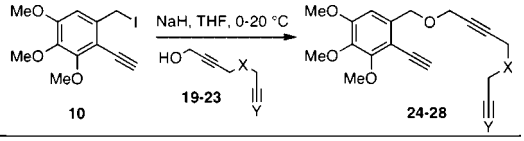
(13) (a) Henry, J. R.; Marcin, L. R.; McIntosh, M. C.; Scola, P. M.; Harris, G. D.; Weinreb, S. M. *Tetrahedron Lett.* **1989**, *30*, 5709–5712. (b) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831–5834.

Scheme 3. Preparation of Cyclization Precursors *rac*-**14**, *rac*-**17**, and *rac*-**18**



zylic iodide **10** with the diynols **19**−**23** (Scheme 4). As the sulfonamide **23** proved to be rather base-sensitive, its alkoxide (NaH, 0 °C) was immediately trapped by addition of **10** to give **28** in at least 40% yield.

Scheme 4. Preparation of Cyclization Precursors **24**−**28**

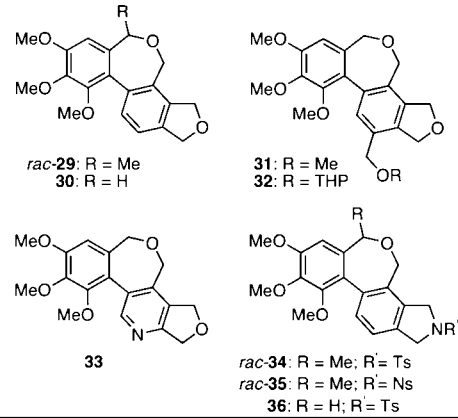


entry	alcohol	X	Y	product (yield)
1	**19**	O	CH	**24** (89%)
2	**20**	O	CCH_2OMe	**25** (94%)
3	**21**	O	CCH_2OTHP	**26** (82%)
4	**22**	O	N	**27** (88%)
5	**23**	NTs	CH	**28** (40%)

Having established reliable entries to various cyclization precursors the stage was set to investigate the key [2 + 2 + 2]-cycloadditions (Scheme 1). As promising catalysts we selected $RhCl(PPh_3)_3$ and $CpCo(CO)_2$.[8] Initial trials under conventional thermal conditions gave only disappointing results, however, the cyclotrimerization reaction proceeded well under microwave (μw) conditions.[14]

The results of various experiments are summarized in Scheme 5. All reactions were performed in sealed microwave vessels in degassed solvents under control of temperature and pressure. As a first substrate, we studied triyne *rac*-**14**, which afforded the product *rac*-**29** in 52% yield upon heating with of $RhCl(PPh_3)_3$ (10 mol %) in toluene (μw, 80 °C, 300 W) for 30 min (conditions A). Using $CpCo(CO)_2$ (20 mol %) as a catalyst and chlorobenzene as a solvent, *rac*-**29** was obtained in 65% within 30 min (μw, 150 °C, 300 W), and the yield could be further improved to 85% by adding PPh_3 (40 mol %) in order to stabilize the cobalt species during the catalytic cycle (conditions B).[15] Under the same conditions (B), triynes *rac*-**17**, **24**, **25**, **26**, and **28** and the cyanodiyne **27** were all smoothly cyclized to give the different allocolchicinoids in typically 70% yield (Scheme 5). Only in the case of the *N*-nosylated substrate *rac*-**18**, the Rh-catalyzed process (conditions A) gave better yields of the product *rac*-**35**, while the corresponding *N*-tosyl compound *rac*-**34** was obtained in 70% yield under co-catalysis (conditions B).

Scheme 5. Key [2 + 2 + 2] Cycloaddition Experiments



entry	substrate	conditions[a]	product	yield
1	*rac*-**14**	A	*rac*-**29**	52%
2	*rac*-**14**	B	*rac*-**29**	85%
3	**24**	B	**30**	90%
4	**25**	B	**31**	77%
5	**26**	B	**32**	51%
6	**27**	B	**33**	71%
7	*rac*-**17**	B	*rac*-**34**	70%
8	*rac*-**18**	A	*rac*-**35**	54%
9	*rac*-**18**	B	*rac*-**35**	25%
10	**28**	B	**36**	65%

[a] **A**: $RhCl(PPh_3)_3$ (10 mol %), toluene, μw (300 W), 30 min, 80 °C, sealed tube. **B**: $CpCo(CO)_2$ (20 mol %), PPh_3 (40 mol %) chlorobenzene, μw (300 W), 30 min, 150 °C, sealed tube.

The tetracyclic structures of the 6-oxa-allocolchicinoids (**29**−**36**) were in accordance with the NMR spectrocopic data, and the assignments were additionally confirmed through X-ray crystal structure analysis of **30**. The structure

(14) (a) Hrdina, R.; Kadlèíková, A.; Valterová, I.; Hodaèová, J.; Kotora, M. *Tetrahedron: Asymmetry* **2006**, *17*, 3185–3191. (b) Young, D. D.; Deiters, A. *Angew. Chem.* **2007**, *46*, 5187–5190. (c) Shanmugasundaram, M.; Aguirre, A. L.; Leyva, M.; Quan, B.; Martinez, L. E. *Tetrahedron Lett.* **2007**, *48*, 7698–7701. (d) Teske, J. A.; Deiters, A. *J. Org. Chem.* **2008**, *73*, 342–345. (e) Mıšek, J.; Teplý, F.; Stará, I. G.; Tichý, M.; Šaman, D.; Císaøová, I.; Vojtıšek, P.; Starý, I. *Angew. Chem.* **2008**, *47*, 3188–3191. (f) Sripada, L.; Teske, J. A.; Deiters, A. *Org. Biomol. Chem.* **2008**, *6*, 263–265.

(15) Stará, I. G.; Starý, I.; Kollárovic, A.; Teplý, F.; Šaman, T. *J. Org. Chem.* **1998**, *63*, 4046–4050.

of **30** shows a conformational twist of ca. 44° along the biaryl axis (Figure 2).

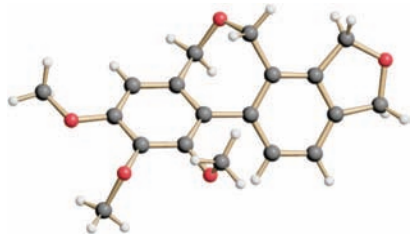


Figure 2. Structure of **30** in the crystaline state.

The effect of allocolchicinoids *rac*-**29**, **30**, **31**, **32a**,[16] and **33** on BJAB tumor cells (Burkitt-like lymphoma cells) was then studied in vitro. It was found that *rac*-**29**, **30**, and **31** significantly inhibit proliferation and induce apoptosis at lower micromolar concentrations, whereas **32a** and **33** proved to be little or not active (Table 1).[17] In the range of good efficacy (AC_{50}) the allocolchicinoids *rac*-**29**, **30**, and **31** exhibited only low cytotoxicity after 1 h (as determined by LDH release).

Table 1. Apoptosis-Inducing Activity of New Allocolchicinoids[a]

entry	allocolchicinoid	apoptosis induction (AC_{50})[b]
1	*rac*-**29**	60 μM
2	**30**	10 μM
3	**31**	40 μM
4	**32a**	na[c]
5	**33**	na[c]

[a] Apoptotic cell death was determined after 72 h by a modified cell cycle analysis, which detects DNA fragmentation on the single cell level (see Supporting Information). For measurement of DNA fragmentation cells were seeded at a density of 1×10^5 cells/mL and treated with different concentrations of the allocolchicinoids ranging from 10 to 100 μM. [b] AC_{50}: 50% apoptotic cells in culture. [c] na = not achieved; 100 μM of **32a** induced 31% apoptosis, 100 μM of **33** induced 3% apoptosis.

Figure 3 illustrates the induction of apoptosis in BJAB cells induced by **30**, i.e., the most active one of the compounds investigated. At 10 μM 50% of the cells showed the DNA damage typical for apoptosis, and nearly all cells died at a concentration of 20 μM (Figure 4).[18]

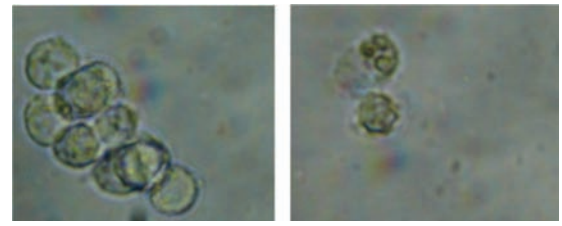


Figure 3. Morphology of BJAB cells (×500): (left) untreated; (right) apoptotic cells after treatment with **30** (20 μM) for 48 h.

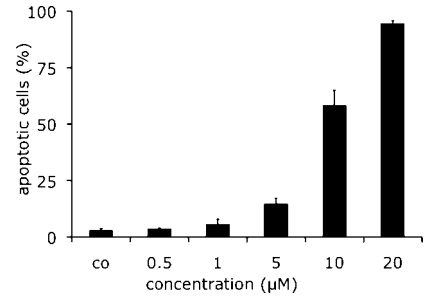


Figure 4. Apoptosis of lymphoma cells (BJAB) induced by different concentrations of **30** after 72 h.

In conclusion, we have elaborated a particularly short (six linear steps) and efficient synthetic route to a new class of tetracyclic 6-oxa-allocolchicinoids exploiting a microwave-induced metal-catalyzed intramolecular [2 + 2 + 2]-cycloaddition. In the key step a remarkable amount of molecular complexity (three new rings, including the twisted seven-membered ring) is efficiently generated in one single step. The methodology paves the way to a rather broad variety of new allocolchicinoids. Moreover, first biological data suggest that this new class of allocolchicinoids represents a promising lead for further investigations. Current studies focusing on the induction of apoptosis in multiple-drug-resistant cells, and tumor reduction in vivo will be communicated separately in due course.

Acknowledgment. This work was supported by the Volkswagenstiftung.

Supporting Information Available: Details regarding preparation, characterization, and biological data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Compound **32a** (R = H) was obtained in 88% yield from **32** by treatment with catalytical amounts of *p*-TsOH in methanol.

(17) Apoptotic cell death was determined by a modified cell cycle analysis, which detects DNA fragmentation on the single cell level (see Supporting Information); see also: Wieder, T.; Prokop, A.; Bagci, B.; Essmann, F.; Bernicke, D.; Schulze-Osthoff, K.; Dörken, B.; Schmalz, H.-G.; Daniel, P. T.; Henze, G. *Leukemia* **2001**, *15*, 1735–1742.

(18) The involvement of the mitochondria as an early sign of apoptosis was detected in cells treated with **30** by measuring the mitochondrial membrane potential $\Delta\Psi_m$ (see Supporting Information).