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Structure–Activity Relationships of Halichondrin B Analogues: Modifications at C.30–C.38

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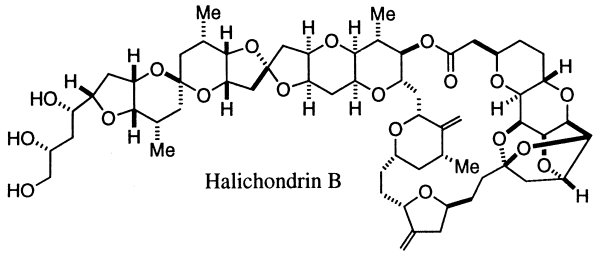
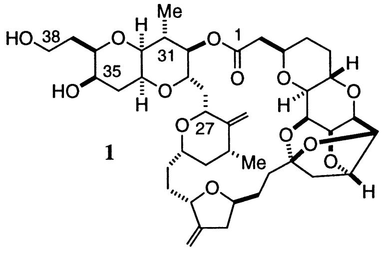
Abstract—Structurally simplified analogues of halichondrin B were prepared by total synthesis and found to retain potent cell growth inhibitory activity in vitro. © 2000 Elsevier Science Ltd. All rights reserved.

Halichondrin B (HB) is a polyether macrolide that was isolated from several marine sponges[1] and found to exhibit extraordinary cytotoxic activity in vitro and potent anticancer activity in vivo.[2] Biochemical characterization demonstrates that HB inhibits tubulin polymerization and microtubule assembly[3] resulting in disruption of mitotic spindle formation and induction of mitotic arrest.

The potential for HB to become a clinically important anticancer drug,[4] its scarcity from natural sources, the need for larger quantities to support drug development research, as well as its challenging structural architecture prompted a number of research groups to undertake a total synthesis of the natural product.[5] The first and to-date only successful effort was reported by Kishi et al. in 1992.[6] Subsequent improvements in their preparation of the C.1–C.12,[7] C.1–C.13,[8] C.14–C.38[9] and C.27–C.38[10] fragments have since been reported.



Halichondrin B



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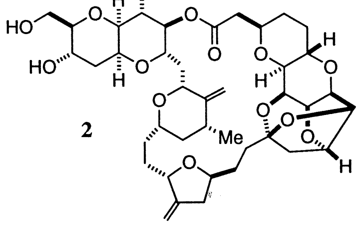

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Many of the intermediates prepared in connection with the Kishi group's total synthesis were evaluated in vitro for cell growth inhibitory activity. Biological activity was found with the macrocyclic lactone fragment **1** (C.1–C.38 fragment, halichondrin numbering),[9,11,12] which demonstrated for the first time that substantially smaller substructure-based analogues of the natural product could furnish potent biologically active compounds.[13] Compound **2** was also prepared by the Kishi group[14] and found to exhibit in vivo antitumor activity in the LOX human melanoma xenograft model.[11] Herein we report our structure–activity relationship (SAR) studies on the C.30–C.38 fragment of HB (Scheme 1).

Macrolactone **1** and its derivatives represented generically by **6** were assembled in a highly convergent man-

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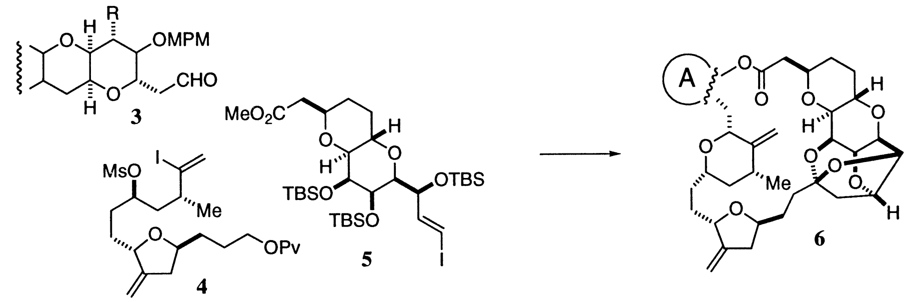
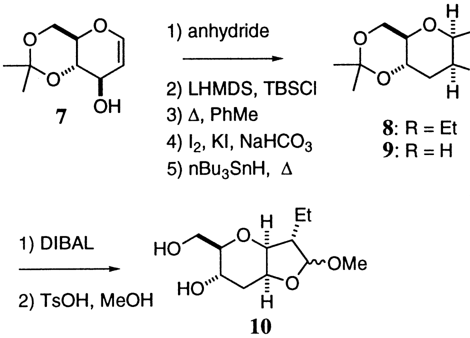


Scheme 1. Key fragments.



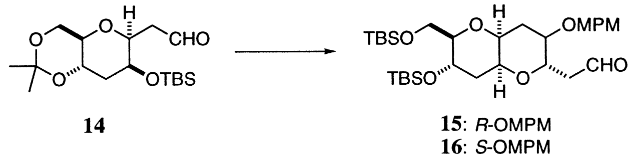
Scheme 2.

ner from three key fragments **3**, **4**, and **5** analogous to that described by Kishi et al.[6] in their total synthesis of HB. Fragments **4** and **5** were prepared as previously described.[6] Aldehydes represented generically by **3** were prepared using a modified procedure of Kishi as follows: glycal **7**[15] was acylated using the appropriate anhydride and converted to lactones **8** and **9**. The desired C.31 ethyl isomer was isolated by column chromatography following iodolactonization and iodide removal. Sequential treatment of **8** with DIBAL and TsOH/MeOH furnished methyl acetal **10** (Scheme 2).

Conversion to aldehyde **11** under standard conditions, Nozaki–Hiyama–Kishi coupling,[16] isomer separation and MPM ether formation furnished acrylate **12**. Following a modified procedure of Kishi, exhaustive desilylation of **12** occurred with concomitant intramolecular ring closure. Elaboration subsequently furnished aldehyde **13** (Scheme 3).

Aldehydes **15** and **16** were prepared from lactone **9** via **14** using a similar series of transformations (Scheme 4).

Aldehydes **13**, **15**, and **16** were converted to macrolactones **22**, **23**, and **24**, respectively, as previously described for the total synthesis of HB.[6]



Scheme 4.

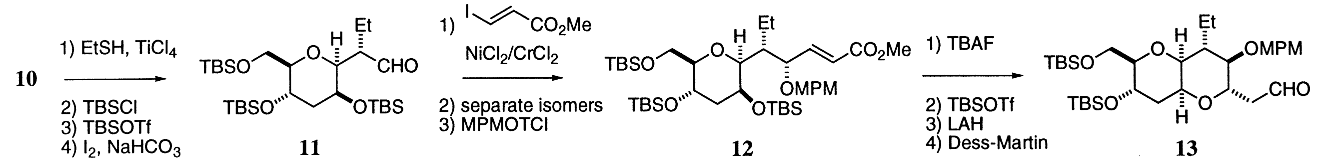
Derivatives **17–21** were prepared from compound **1** using standard transformations (Scheme 5).

These compounds were evaluated for cell growth inhibitory activity against DLD-1 human colon cancer cells under continuous exposure conditions, the ability to maintain a mitotic block 10 h after drug washout using flow cytometric analysis of U937 human histiocytic lymphoma cells,[17] and susceptibility to P-glycoprotein (PgP) mediated drug efflux using murine P388/VMDRC.04 cells, a multidrug resistant (MDR) subline of murine P388/S leukemia cells.[18]

As summarized in Table 1, there were no remarkable intrinsic potency differences between analogues **17–21** that would distinguish them from each other or from lead compound **1**. Thus, the structural requirements for inhibiting human cancer cell growth under continuous exposure conditions (DLD-1 assay) are more liberal than those for inducing an irreversible mitotic block after drug washout (U937 mitotic block reversibility assay). For example, homologation of the C.36 alkoxyl chain of **2** by one carbon (e.g., **21**) significantly reduced

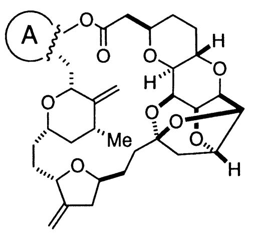
$$\begin{tikzcd}
\mathbf{17} & \mathbf{1} \arrow[l, "a"'] \arrow[r, "e"] \arrow[dl, "b"'] \arrow[d, "c"] \arrow[dr, "d"] & \mathbf{21} \\
\mathbf{18} & \mathbf{19} & \mathbf{20}
\end{tikzcd}$$

Scheme 5. TsCl, pyridine; (b) i. TBSCl, ii. Swern, iii. TBAF; (c) Dess–Martin; (d) NaH, MeI; (e) i. TBSCl, ii. Mitsunobu, iii. TBAF.



Scheme 3.

Table 1. In vitro activity profile for compounds of generic structure **6**



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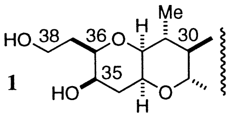

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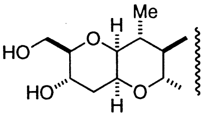

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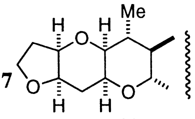

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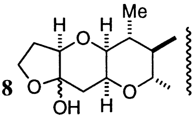

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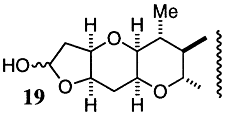

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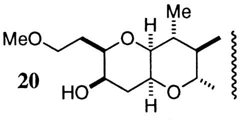

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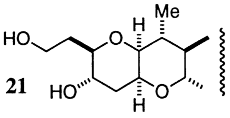

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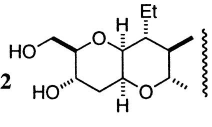

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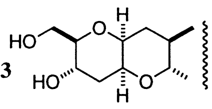

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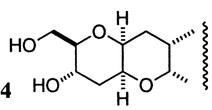

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No.	DLD-1[a]	U937[b]	P388/S[a]	P388/VMDRC.04[a]	FR[c]
Paclitaxel	27±4 (8)	230 (3)	16±0.6 (3)	170±20 (3)	11
HB	0.74±0.03 (6)	25 (4)	0.60±0.1 (6)	1.7±0.1 (6)	2.8
1	4.6±0.2 (12)	>880 (2)	2.2±0.3 (9)	8.8±0.8 (9)	4.0
2	3.4±0.4 (4)	220 (6)	0.71±0.05 (7)	8.2±0.6 (7)	12
17	2.6±0.6 (11)	>1000 (2)	1.3±0.3 (3)	2.7±0.3 (3)	2.1
18	5.2±0.0 (3)	1000 (2)	3.1 (2)	6.3 (2)	2.0
19	2.1±0.2 (4)	>1000 (2)	1.5 (2)	3.8 (2)	2.5
20	1.2±0.1 (3)	>650 (2)	0.55 (2)	1.5 (2)	2.7
21	3.6±0.1 (3)	>1000 (2)	1.7 (2)	16 (2)	9.4
22	6.0±0.9 (3)	1000 (2)	1.6 (2)	16 (2)	10
23	610±70 (3)	ND[d]	ND[d]	ND[d]	ND[d]
24	230±10 (3)	ND[d]	ND[d]	ND[d]	ND[d]

[a] Cell growth inhibition under continuous exposure conditions for 3–4 days, IC_{50}±SEM nM (n).

[b] Mitotic block reversibility assay. Cells were treated with test compound for 12 h followed by a 10 h drug washout, IC_{99} nM (n).

[c] Fold resistance calculated as the IC_{50} ratio between retrovirally transformed P388/VMDRC.04 and parental P388/S cells.

[d] Not determined.

activity in the U937 mitotic block reversibility assay, without impacting intrinsic potency (compare **1** and **21** with compound **2**). Structure modifications to this region of the molecule also altered susceptibility to PgP-mediated drug efflux as indexed by the FR ratio (cf. **2** and **17**).

Increasing the size of the C.31 substituent from methyl to ethyl had only a modest negative effect on in vitro potency. Removing the methyl group altogether, however, reduced activity over two orders of magnitude. Inverting the vicinal C.30 stereocenter as in **24** only marginally helped restore in vitro potency.

Current evidence suggests that these compounds, like HB, bind to either both α and β tubulin monomers or to α/β tubulin heterodimers, which in turn disrupts mitotic spindle formation with subsequent induction of cell cycle arrest.[11] It is not clear at the present time if compound **2** simply binds more tightly to its macromolecular target with a reduction in off rate or if the structural change simply allows the compound to be more efficiently retained within the cell. Either possibility would explain the increased ability of **2** to maintain a mitotic block 10 h after drug washout.

Thus, although many of the compounds in this series inhibited DLD-1 human colon cancer cell growth at low nanomolar concentrations in vitro, compound **2** was the most efficacious in maintaining a mitotic cell block after drug washout and therefore exhibited the most favorable in vitro profile.

Acknowledgements

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References and Notes

1. (a) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701. (b) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.;

Hooper, J. N. A.; Rützler, K. C. *J. Med. Chem.* **1991**, *34*, 3339. (c) Litaudon, M.; Hart, J. B.; Blunt, J. W.; Lake, R. J.; Munro, M. H. G. *Tetrahedron Lett.* **1994**, *35*, 9435.

2. Fodstad, Ø.; Breistøl, K.; Pettit, G. R.; Shoemaker, R. H.; Boyd, M. R. *J. Exp. Therap. Oncol.* **1996**, *1*, 119.

3. (a) Hamel, E. *Pharmacol. Ther.* **1992**, *55*, 31. (b) Bai, R.; Paull, K. D.; Herald, C. L.; Malspeis, L.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1991**, *266*, 15882. (c) Luduena, R. F.; Roach, M. C.; Prasad, F.; Pettit, G. R. *Biochem. Pharmacol.* **1993**, *45*, 421.

4. Halichondrin B is under preclinical development by the United States National Cancer Institute, Developmental Therapeutics Program. See, for example Proceedings for the 89th Annual Meeting of the American Association for Cancer Research, abstr. #1544, #1545, 1998.

5. See, for example: (a) Horita, K.; Nagasawa, M.; Hachiya, S.-I.; Sakurai, Y.; Yamazaki, T.; Uenishi, J.; Yonemitsu, O. *Tetrahedron Lett.* **1997**, *38*, 8965. (b) Horita, K.; Sakurai, Y.; Nagasawa, M.; Yonemitsu, O. *Chem. Pharm. Bull.* **1997**, *45*, 1558. (c) Horita, K.; Hachiya, S.-I.; Yamazaki, T.; Naitou, T.; Uenishi, J.; Yonemitsu, O. *Chem. Pharm. Bull.* **1997**, *45*, 1265. (d) Burke, S. D.; Philips, J. R.; Quinn, K. J.; Zhang, G.; Jung, K. W.; Buchanan, J. L.; Perri, R. E. *Spec. Publ.-R. Soc. Chem.* **1997**, *198*, 73. (e) Horita, K.; Hachiya, S.-I.; Ogihara, K.; Yoshida, Y.; Nagasawa, M.; Yonemitsu, O. *Heterocycles* **1996**, *42*, 99. (f) Burke, S. D.; Zhang, G.; Buchanan, J. L. *Tetrahedron Lett.* **1995**, *36*, 7023. (g) Burke, S. D.; Jung, K. W.; Phillips, J. R.; Perri, R. E. *Tetrahedron Lett.* **1994**, *35*, 703. (h) Horita, K.; Sakurai, Y.; Nagasawa, M.; Hachiya, S.; Yonemitsu, O. *Synlett* **1994**, 43. (i) Horita, K.; Sakurai, Y.; Nagasawa, M.; Maeno, K.; Hachiya, S.; Yonemitsu, O. *Synlett* **1994**, 46. (j) Horita, K.; Nagasawa, M.; Hachiya, S.; Yonemitsu, O. *Synlett* **1994**, 40. (k) Cooper, A. J.; Pan, W.; Salomon, R. G. *Tetrahedron Lett.* **1993**, *34*, 8193. (l) Horita, K.; Hachiya, S.; Nagasawa, M.; Hikota, M.; Yonemitsu, O. *Synlett* **1994**, 38. (m) DiFranco, E.; Ravikumar, V. T.; Salomon, R. G. *Tetrahedron Lett.* **1993**, *34*, 3247. (n) Fang, F. G.; Kishi, Y.; Matelich, M. C.; Scola, P. M. *Tetrahedron Lett.* **1992**, *33*, 1557. (o) Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Scola, P. M.; Yoon, S. K. *Tetrahedron Lett.* **1992**, *33*, 1553. (p) Burke, S. D.; Buchanan, J. L.; Rovin, J. D. *Tetrahedron Lett.* **1991**, *32*, 3961. (q) Cooper, A. J.; Salomon, R. G. *Tetrahedron Lett.* **1990**, *31*, 3813. (r) Kim, S.; Salomon, R. G. *Tetrahedron Lett.* **1989**, *30*, 6279. (s) Aicher, T. D.; Kishi, Y. *Tetrahedron Lett.* **1987**, *28*, 3463.

6. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162.

7. Duan, J. J. W.; Kishi, Y. *Tetrahedron Lett.* **1993**, *34*, 7541.

8. Stamos, D. P.; Kishi, Y. *Tetrahedron Lett.* **1996**, *37*, 8643.

9. Stamos, D. P.; Sean, S. C.; Kishi, Y. *J. Org. Chem.* **1997**, *62*, 7552.

10. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Scola, P. M. *Tetrahedron Lett.* **1992**, *33*, 1549.

11. Unpublished results, Eisai Research Institute.

12. Kishi, Y.; Fang, F. G.; Forsyth, C. J.; Scola, P. M.; Yoon, S K. U.S. Patent 5,436,238.

13. Since that time, certain polyether fragments of HB have been reported to exhibit cytotoxic activity against KB and L1210 cells in vitro. Horita, K.; Nishibe, S.; Yonemitsu, O. *Int. Congr. Ser.* **1998**, *1157* (Towards Natural Medicine Research in the 21st Century), 327.

14. Manuscript in preparation.

15. Casillas, M.; Gómez, A. M.; López, J. C.; Valverde, S. *Synlett* **1996**, 628.

16. (a) Jin, H; Uenishi, J.; Christ, W.J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644. (b) Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048. (c) Kishi, Y. *Pure Appl. Chem.* **1992**, *64*, 343. (d) Cintas, P. *Synthesis* **1992**, 248.

17. Littlefield, B. A.; Palme, M. H.; Seletsky, B. M.; Towle, M. J.; Yu, M. J.; Zheng, W. U.S. Patent Application 09/334,488.

18. Yang, J.-M.; Goldenberg, S.; Gottesman, M. M.; Hait, W. N. *Cancer Res.* **1994**, *54*, 730.