Synthesis of Antitumor Agents

Total Synthesis as a Resource in the Discovery of Potentially Valuable Antitumor Agents: Cycloproparadicicol**

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Hsp90 is a molecular chaperone required for the refolding of proteins in cells exposed to environmental stress. It governs the conformational maturation of a subset of proteins critical in regulating mitotic signal transduction and cellular survival.[1] These client proteins include steroid receptors, transcription factors, mutant p53, Hif1α, soluble kinases such as Akt, Raf-1, transmembrane kinases such as HER2, as well as cdk4 protein kinases. Hsp90 contains an ATP-binding pocket in its amino terminus. Several natural products, including geldanamycin, herbimycin A, and radicicol bind to this pocket and inhibit its chaperone function. This inhibition is mirrored in enhanced intracellular proteasomal degradation of Hsp90 client proteins. An encouraging consequence of this effect is an attenuation in the proliferation of cancer cells. In animal models, geldanamycin congeners exhibit antitumor activity at nontoxic doses.[2] The novel mechanism of action of geldanamycin-related drugs and the demonstration that they have, in principle, exploitable in vivo therapeutic indices have served to heighten interest in Hsp90 as a target for oncology.[3,4]

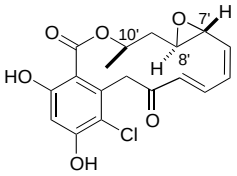
Radicicol (**1**),[5] originally isolated from *M. bonorden*, has a high affinity for Hsp90 ($K_d = 20$ nM) and is particularly potent in inhibiting its functions.[6] However, translation of the very promising in vitro profiles of radicicol and radicicol oxime to the in vivo realm has not been accomplished,[7] apparently as a result of unfavorable pharmakokinetics and nonspecific toxicities.

These early findings notwithstanding, we felt it worthwhile to persist in seeking a useful radicicol-based drug that is not subject to the potential liabilities arising from quinone-containing substructures such as those found in the geldanamycin series. We previously developed a highly convergent, three-stage total synthesis of radicicol,[8] which was crucial to the work described herein.

Our investigation of the radicicol series focused on two aspects. The first was directed to the effect, if any, of the stereochemistry of the system on its biological properties. We hoped to examine binding to Hsp90 as well as cytotoxicity. We saw such a study as a way to probe the areas of the drug that make important contacts with the chaperone. Assessing the consequences of altering the stereochemistry at particular points in a complex drug may be a more subtle probe of the stringency of presumably required molecular contacts, than gross deletion of functional groups.

We also became interested in a cyclopropane derivative of radicicol, that is, one in which the oxido linkage that connects C7′ and C8′ is replaced by a methylene group (i.e., a cyclopropane analogue, see Scheme 4, **21**–**24**).[9,10] The thought was that the lack of in vivo efficacy of radicicol might arise from its degradation ($k_{1/2} < 2$ hrs) or undirected cytotoxicity emanating from the ω-epoxydienone. Given the structural information available[11] on the binding of radicicol to the N-terminal ATP/ADP-binding domain of Hsp90, which seems to identify a hydrogen bond between the epoxide and Lys44, there was concern that the cyclopropane derivative might suffer an unacceptable loss of affinity to its intended target. In essence, both aims of this inquiry focused on a careful assessment of Lys44 contact in the action of the drug.

Our previous total synthesis of **1** provided a method for the preparation of compounds **2**–**4**. As before, the synthesis



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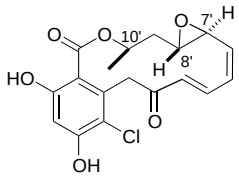

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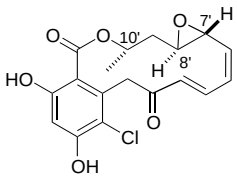

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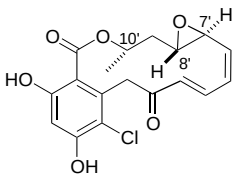

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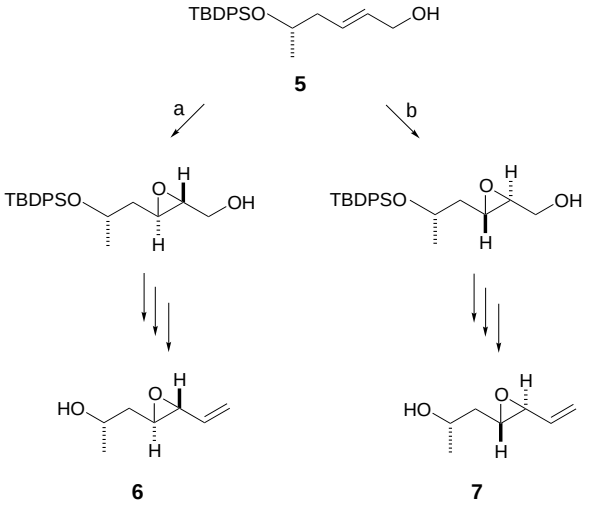
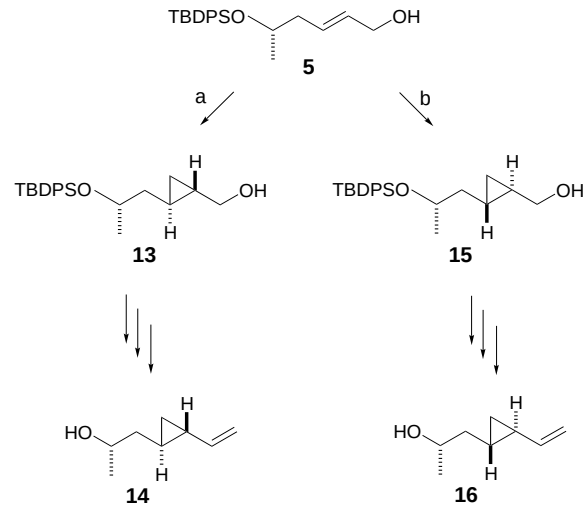
featured a novel ring-closing olefin metathesis of a vinyl epoxide.[12,13] Thus, alcohol **5** was converted into **6** or **7** by the sequence described earlier (Scheme 1).[8] Coupling of **6** with acid **8** under either Mitsunobu (C10′ inversion) or acylation (C10′ retention) experimental protocols afforded esters **9** and **11**, respectively. Similarly, when the two senses of coupling were applied to alcohol **7**, esters **10** and **12** were obtained. The strategy used in the total syntheses of radicicol allowed access to stereocongeners **2, 3** (*ent*-**2**), and **4** (*ent*-**1**), as shown in Scheme 2.

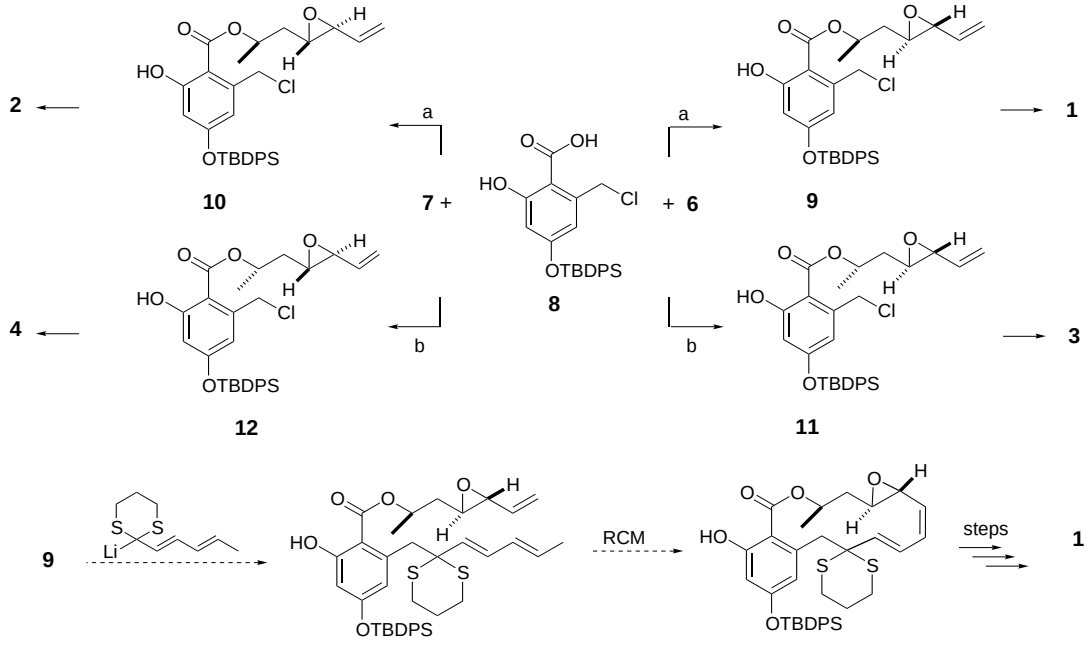
The syntheses of the cyclopropane analogues commenced with the same allylic alcohol precursor **5** used in our earlier synthesis of radicicol and its stereocongeners. We now took advantage of the asymmetric cyclopropanation chemistry reported by Charette et al.[14,15] for the conversion of **5** into **13**. Alcohol **13** was converted into vinyl compound **14** (Scheme 3). Again acylation of **14** with **8** by using the two stereodifferentiating protocols led to **17** and **19** in high enantiomeric and diastereomeric purity. The conversion of **17** into the carba version of radicicol (cf. **21**) was carried out as shown. Conversion of **19** into **23** followed an identical route (Scheme 4).



Scheme 1. Synthesis of the enantiopure epoxide side chains. a) (*R,R*)-diisopropyltartrate, $TiCl_4$, CH_2Cl_2, molecular sieves (4 Å), 71%; b) (*S,S*)-diisopropyltartrate, $TiCl_4$, molecular sieves (4 Å), 76%. TBDPS = *tert*-butyldiphenylsilyl.



Scheme 3. Synthesis of the enantiopure cyclopropyl side chains. a) (*R,R*)-dioxaborolane, Et_2Zn, CH_2I_2, 69%; b) (*S,S*)-dioxaborolane, Et_2Zn, CH_2I_2, 72%.



Scheme 2. Divergent synthesis of radicicols. a) DIAD, $P(fur)_3$, benzene, 75% (**9**), 76% (**10**); b) EDCI, DMAP, CH_2Cl_2, 41% (**11**), 44% (**12**). DIAD = diisopropylazodicarboxylate, fur = furyl, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, DMAP = 4-dimethylaminopyridine, RCM = ring-closing olefin metathesis.

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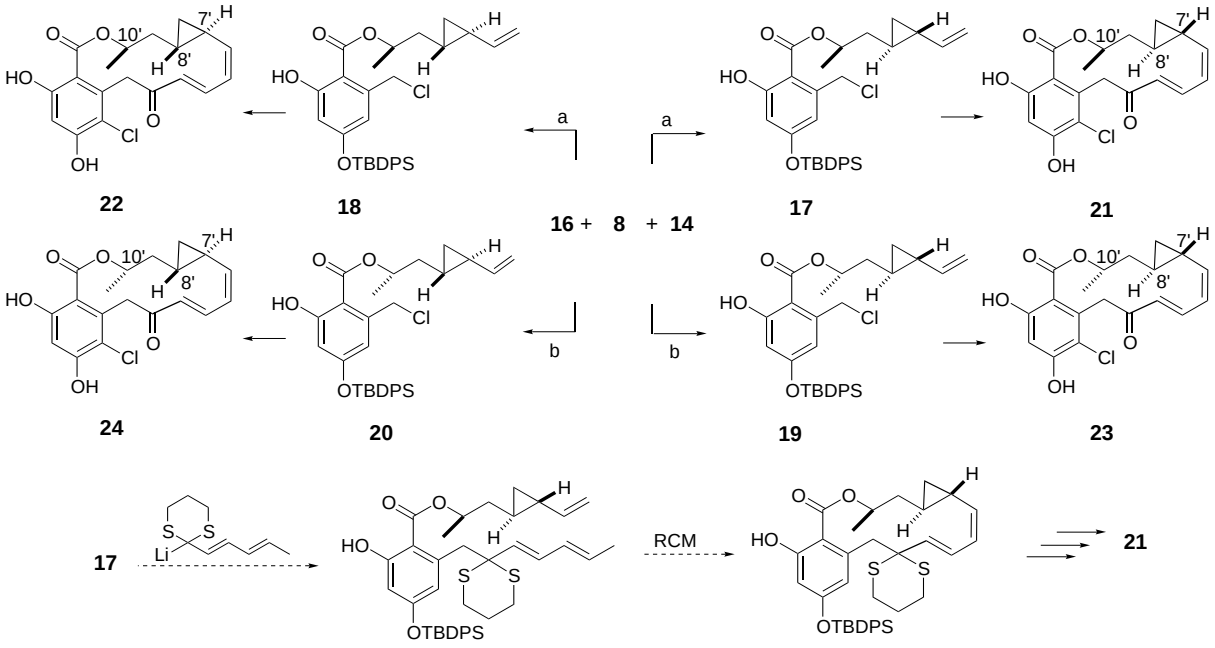

Scheme 4. Divergent synthesis of cycloproparadicicols. a) DIAD, $P(fur)_3$, benzene, 48% (**17**), 39% (**28**); b) EDCI, DMAP, CH_2Cl_2, 54% (**19**), 61% (**20**).

In a similar way **16** was obtained from **5** by using the enantiotopic Charette catalyst (Scheme 3). The conversion of **16** into **18** and **20** followed the same pathway. These, in turn, gave rise to **22** and **24**, respectively, again following closely the strategy and protocols used to reach **21**. Thus the four radicicol stereocongeners **1**–**4** as well as their respective carba variants **21**–**24** became available.

The radicicol congeners were tested for binding with Hsp90 by means of a binding-competition assay.[4] The parent natural product **1** binds with high affinity to Hsp90 (ED_{50} = 45 nM) as reported previously. When **21**, the carba version of the radicicol series, was tested for Hsp90 binding, it was found to still retain high affinity (ED_{50} = 160 nM), that is, a fourfold reduction from **1**. Therefore the hydrogen bond found between the epoxide and the Lys44 of the chaperone in the X-ray structure, while possibly useful, is not critical. Compound **22**, in which the C7′ and C8′ stereocenters are inverted, exhibits binding to a much lower extent (ED_{50} = 2 μM). Compound **23** with inversion at the C10′ stereocenter suffered a further decrease in its binding (ED_{50} = 5 μM). Inversion of all these stereocenters (see **24** = *ent*-**21**), undermines the affinity to Hsp90 still further (ED_{50} = > 10 mM).[16] It would be tempting to argue that in the cycloproparadicicols, and probably in the radicicol series, the protein Hsp90 target recognizes the gross substructure of the 14-membered lactone fused to the resorcinol. Supporting recognition elements are found in the methyl group at C10′ and at the cyclopropyl (epoxide) group, in the "natural" series when these elements are in an optimal stereochemical setting. Inversion of either of these loci, diminishes binding and biological activity. Inversion at both of the supporting sectors leads to severe loss of recognition.

As previously reported, the inhibition of Hsp90 induces the proteasomal degradation of a subset of proteins that require Hsp90 for maturation or stability. The expression levels of Raf1 and HER2, two of the most sensitive protein targets, was analyzed with the above radicicol variants (Figure 1). The radicicol-like action of **21** is also reflected in its potent degradation of oncogenic proteins. Interestingly, the stereochemical trend found in the Hsp90-binding assay, was also observed in the degradation level of the oncogenic proteins in both series. It seems that the actions of **1** and **21** on Hsp90 appear to occur in the same recognition setting.

Subsequently, these radicicol variants were evaluated for their growth-inhibition activity towards MCF-7 breast-cancer cells (Table 1). As expected, the same configurational trend

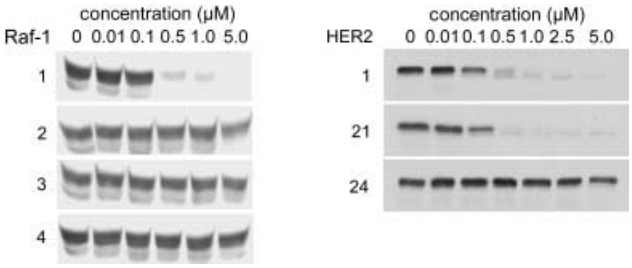

Figure 1. Immunoblot of Raf1 and HER2 expression of MCF-7 cells following 24 h of exposure to the indicated drugs.

Table 1: IC_{50} (nM) values of radicicol and cycloproparadicicol analogues in MCF7 cells.

Entry	Epoxide	IC_{50} [nM][a]	Cyclopropyl	IC_{50} [nM][a]
1	**1**	23	**21**	43
2	**2**	902	**22**	836
3	**3**	1720	**23**	2142
4	**4**	2172	**24**	3364

[a] Determined after 96 h of exposure to the indicated drugs.

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was also observed in this assay. Thus, **2** exhibits intermediate cell-growth inhibition (20-fold less), whereas **3** showed further decrease in their potency (50-fold less), and **4** was found to be even less active (~100-fold less). Most significantly, cyclopropane analogue **21**, which has the same stereochemical motif as radicicol (**1**), *retains* the potent cytotoxicity of **1** within a factor of two.

As previously reported for ansamycins, growth inhibition by radicicol (**1**) is a result of an Rb-dependent (Rb = retinoblastoma) arrest in the G1 phase of the cell cycle (Figure 2). Thus, MDA-468 breast cancer, which lacks Rb function, does

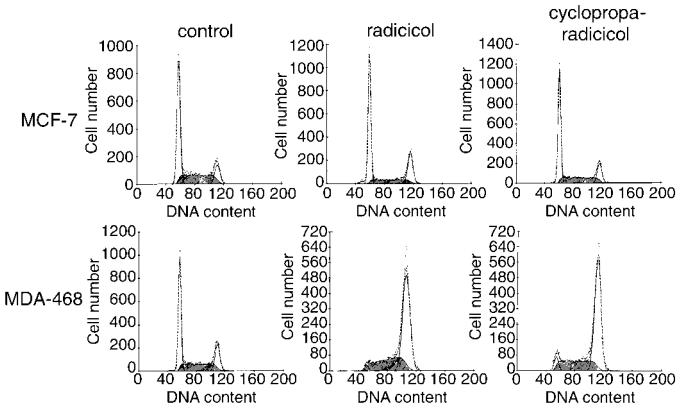

Figure 2. Cell cycle distribution of MCF-7 or MDA-468 cell lines assessed by fluorescence-activated cell sorter (FACS) analysis.

not arrest in G1, but instead is blocked in prometaphase prior to undergoing apoptosis.[17] Inhibition of Rb-dependent growth occurred at concentrations that correlate with those required for protein degradation. Cells with intact Rb (MCF-7) arrested in G1 and then underwent differentiation. Importantly, Rb-negative cells (MDA-468) also underwent apoptosis, in this case upon arrest in prometaphase. The same propensity was observed with cyclopropradicicol **21**.

In summary, these stereo and "carba" radicicol variants were designed and first prepared in our laboratory following the outlines of our concise total synthesis of radicicol.[8] The in vitro biological testing of these compounds revealed previously unrecognized structure–activity relationships. Notably, the oxygen atom of the epoxide of radicicol (**1**), in spite of its apparent Lys44–epoxide hydrogen bonding in the governing crystal structure in the radicicol field, is demonstrated to be non-essential for its activity.[18] A qualitative though instructive recognition model can now be proposed to focus future discovery-oriented investigations in this rather promising series of anticancer agents. The work described above serves to chart a direction by which organic synthesis in conjunction with cell biology can be mutually enhancing in the quest for novel drugs.

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Keywords: antitumor agents · chaperone proteins · natural products · structure–activity relationships · synthesis design

[1] For general reviews of Hsp90, see: a) S. Walter, J. Buchner, *Angew. Chem.* **2002**, *114*, 1142–1158; *Angew. Chem. Int. Ed.* **2002**, *41*, 1098–1113; b) L. H. Pearl, C. Prodromou, *Curr. Opin. Struct. Biol.* **2000**, *10*, 1, 46–51; c) J. Buchner, *Trends Biochem. Sci.* **1999**, *24*, 136–141.

[2] For our efforts involving geldanamycin, another natural product which also binds to Hsp90, see: a) S. D. Kuduk, F. F. Zheng, L. Sepp-Lorenzino, N. Rosen, S. J. Danishefsky, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1233–1238; b) S. D. Kuduk, C. R. Harris, F. F. Zheng, L. Sepp-Lorenzino, Q. Ouerfelli, N. Rosen, S. J. Danishefsky, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1303–1306.

[3] a) L. Neckers, *Trends Mol. Med.* **2002**, *8*, 4, S55-S61; b) M. V. Blagosklonny, *Leukemia* **2002**, *16*(4), 455–462; c) T. Scheibel, J. Buchner, *Biochem. Pharmacol.* **1998**, *56*, 675–682; D. J. Newman, G. M. Cragg, S. Holbeck, E. A. Sausville, *Curr. Cancer Drug Targets* **2002**, *2*, 279–308.

[4] A novel class of synthetic nucleotide analogues was shown to bind to Hsp90 and inhibit the proliferation of cancer cells; see: a) G. Chiosis, M. N. Timaul, B. Lucas, P. N. Munster, F. F. Zheng, L. Sepp-Lorenzino, N. Rosen, *Chem. Biol.* **2001**, *8*, 289–299; b) G. Chiosis, B. Lucas, A. Shtil, H. Huezo, N. Rosen, *Bioorg. Med. Chem. Lett.* **2002**, *10*, 3555–3564.

[5] P. Delmontte, J. Delmontte-Plaquée, *Nature* **1953**, *171*, 344–347.

[6] a) S. V. Sharma, T. Agatsuma, H. Nakano, *Oncogene* **1998**, *16*, 20, 2639–2645; b) T. W. Schulte, S. Akinaga, T. Murakata, T. Agarsuma, S. Sugimoto, H. Nakano, Y. S. Lee, B. B. Simen, Y. Argon, S. Felts, D. O. Toft, L. M. Neckers, S. V. Sharma, *Mol. Endocrinol.* **1999**, *13*(9), 1435–1448.

[7] Some derivatives of microbial radicicol were reported to exhibit efficacy in animal models: a) Y. Shiotsu, L. M. Neckers, I. Wortman, W. G. An, T. W. Schulte, S. Soga, C. Murakata, T. Tamaoki, S. Akinaga, *Neoplasia* **2000**, *96*, 6, 2284–2291; b) S. Soga, S. V. Sharma, Y. Shiotsu, M. Shimizu, H. Tahara, K. Yamaguchi, Y. Ikuina, C. Murakata, T. Tamaoki, J. Kurebayashi, T. W. Schulte, L. M. Neckers, S. Akinaga, *Cancer Chemother. Pharmacol.* **2001**, *48*, 435–445; c) S. Soga, L. M. Neckers, T. W. Schulte, Y. Shiotsu, K. Akasaka, H. Narumi, T. Agatsuma, Y. Ikuina, C. Murakata, T. Tamaoki, S. Akinaga, *Cancer Res.* **1999**, *59*, 2931–2938; d) T. Agatsuma, H. Ogawa, K. Akasaka, A. Asai, Y. Yamashita, T. Mizukami, S. Akinaga, Y. Saitoh, *Bioorg. Med. Chem.* **2002**, *10*, 3445–3454.

[8] a) R. M. Garbaccio, S. J. Danishefsky, *Org. Lett.* **2000**, *2*, 20, 3127–3129; b) R. M. Garbaccio, S. J. Stachel, D. K. Baeschlin, S. J. Danishefsky, *J. Am. Chem. Soc.* **2001**, *123*, 10903–10908.

[9] The electophilicity of this system would allow Michael addition of a bionucleophile to the dienone or attack of the nucleophile at the epoxide linkage.[7b,d] Esterase action on the macrolactone is still another potential site of vulnerability. In the epothilone series, we found that agents lacking the epoxide manifest a much improved biological profile.[10]

[10] a) T. C. Chou, X. G. Zhang, C. R. Harris, S. D. Kuduk, A. Balog, K. Savin, J. R. Bertino, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15798–15802; b) T. C. Chou, X. G. Zhang, A. Balog, D. Su, D. F. Meng, K. Savin, J. R. Bertino, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9642–9647.

[11] S. M. Roe, C. Prodromou, R. O'Brien, J. E. Ladbury, P. W. Piper, L. H. Pearl, *J. Med. Chem.* **1999**, *42*, 260–266.

[12] a) M. S. Stanford, J. A. Love, R. H. Grubbs, *J. Am. Chem. Soc.* **2001**, *123*, 6543–6554, and references therein; b) for reviews, see: A. Fürstner, L. Ackermann, B. Gabor, R. Goddard, C. W. Lehmann, R. Mynott, F. Stelzer, O. R. Thiel, *Chem. Eur. J.* **2001**, *7*, 3236–3253; c) A. Fürstner, *Angew. Chem.* **2000**, *112*, 3140–3172; *Angew. Chem. Int. Ed.* **2000**, *39*, 3012–3043; d) for an important early example of the use of olefin metathesis for macrocyclization in a functionally complex setting, see: Z. Xu,

  1433-7851/03/4211-1283 $ 20.00+.50/0

C. W. Johannes, S. S. Salman, A. H. Hoveyda, *J. Am. Chem. Soc.* **1996**, *118*, 10926 – 10927.
[13] K. Yamamoto, K. Biswas, C. Gaul, S. J. Danishefsky, unpublished results.
[14] a) A. B. Charette, S. Prescott, C. Brochu, *J. Org. Chem.* **1995**, *60*, 1081 – 1083; b) A. B. Charette, H. Juteau, *J. Am. Chem. Soc.* **1994**, *116*, 2651 – 2652; c) A. B. Charette, H. Juteau, H. Lebel, C. Molinaro, *J. Am. Chem. Soc.* **1998**, *120*, 11943 – 11952; d) A. B. Charette, H. Lebel, *Org. Synth.* **1999**, *76*, 86 – 100.
[15] The configuration of the cyclopropane was initially assigned based on Charette's empirical rule and later confirmed by X-ray analysis of the derivative of **14** (see Supporting Information).
[16] This trend is consistent with molecular modeling (MOE 2001.01) results (Chiosis, unpublished data).
[17] Importantly, the small-cell lung cancer, which is not effected by geldanamycin variant are found to be Rb-negative.
[18] On the other hand, other hydrogen bonds in X-ray data appear to be critical as the dimethyl ethers of **1** and its derivatives lost their activities (data not shown).

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