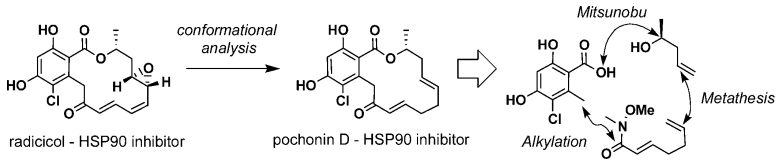
Article

Design, Synthesis, and Biological Evaluation of HSP90 Inhibitors Based on Conformational Analysis of Radicicol and Its Analogues

Emilie Moulin, Vincent Zoete, Sofia Barluenga, Martin Karplus, and Nicolas Winssinger

J. Am. Chem. Soc., **2005**, 127 (19), 6999-7004• DOI: 10.1021/ja043101w • Publication Date (Web): 21 April 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 8 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 04/21/2005

Design, Synthesis, and Biological Evaluation of HSP90 Inhibitors Based on Conformational Analysis of Radicicol and Its Analogues

Emilie Moulin,[†] Vincent Zoete,[‡] Sofia Barluenga,[†] Martin Karplus,*[,‡,§] and Nicolas Winssinger*[,†]

Contribution from the Organic and Bioorganic Chemistry Laboratory, Institut de Science et Ingénierie Supramoleculaires, Université Louis Pasteur, 8 allée Gaspard Monge, 67000 Strasbourg, France, Biophysical Chemistry Laboratory, Institut de Science et Ingénierie Supramoleculaires, Université Louis Pasteur, 8 allée Gaspard Monge, 67000 Strasbourg, France, and Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138

Received November 16, 2004; E-mail: marci@tammy.harvard.edu; winssinger@isis.u-strasbg.fr

Abstract: The molecular chaperone HSP90 is an attractive target for chemotherapy because its activity is required for the functional maturation of a number of oncogenes. Among the know inhibitors, radicicol, a 14-member macrolide, stands out as the most potent. A molecular dynamics/minimization of radicicol showed that there were three low energy conformers of the macrocycle. The lowest of these is the bioactive conformation observed in the cocrystal structure of radicicol with HSP90. Corresponding conformational analyses of several known analogues gave a good correlation between the bioactivity and the energy of the bioactive conformer, relative to other conformers. Based on this observation, a number of proposed analogues were analyzed for their propensity to adopt the bioactive conformation prior to synthesis. This led to the identification of pochonin D, a recently isolated secondary metabolite of *Pochonia chlamydosporia,* as a potential inhibitor of HSP90. Pochonin D was synthesized using polymer-bound reagents and shown to be nearly as potent an HSP90 inhibitor as radicicol.

Introduction

The heat shock protein 90 (HSP90) is an ATP-dependent chaperone whose activity is required for the functional maturation of a number of overexpressed oncogenic proteins that promote the growth or survival of cancer cells.[1−3] The list of HSP90's clients include notorious oncogenes such as Bcr-Abl, Raf1, ErbB2, AKT, and mutated p53. In the absence of this chaperoning activity, HSP90's clients are targeted for degradation by the proteosome. The prospect of stalling a number of oncogenes by inhibiting a single protein has made HSP90 an attractive target for chemotherapy.[4,5] Two natural products, radicicol (**1**, Figure 1) and geldanamycin (**2**, Figure 1), are known to be potent inhibitors of HSP90's chaperone activity. Cocrystal structures of both of these compounds with HSP90 showed that, while neither compound has structural or topological similarity to ATP, they both bind in the ATP-binding pocket of HSP90; their measured K_d values are 19 nM and 1.6 μM, respectively.[6] Designed inhibitors based on an adenosine scaffold led to the discovery of new leads such as **4** (Figure 1).[7−9] More recently, novobiocin was also shown to inhibit HSP90 albeit at millimolar concentration and through a different binding

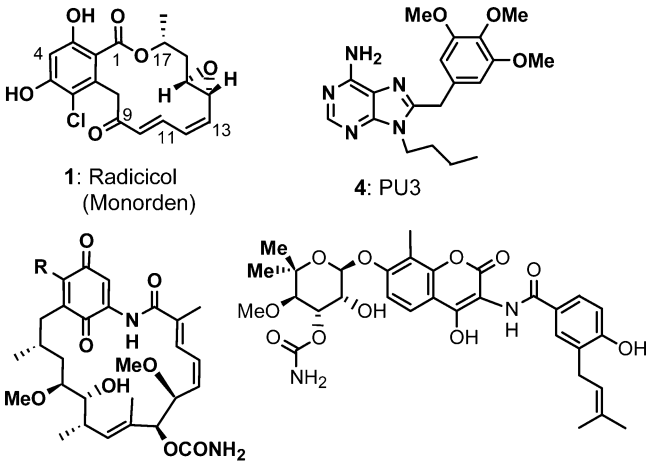


Figure 1. Structures of HSP90 inhibitors.

[†] Organic and Bioorganic Chemistry Laboratory, Institut de Science et Ingénierie Supramoleculaires, Université Louis Pasteur.
[‡] Biophysical Chemistry Laboratory, Institut de Science et Ingénierie Supramoleculaires, Université Louis Pasteur.
[§] Department of Chemistry and Chemical Biology, Harvard University.

(1) Isaacs, J. S.; Xu, W.; Neckers, L. *Cancer Cell* **2003**, *3*, 213−217.
(2) Mosser, D. D.; Morimoto, R. I. *Oncogene* **2004**, *23*, 2907−2918.
(3) Prodromou, C.; Pearl, L. H. *Curr. Cancer Drug Targets* **2003**, *3*, 301−323.
(4) Dymock, B. W.; Drysdale, M. J.; McDonald, E.; Workman, P. *Expert Opin. Ther. Pat.* **2004**, *14*, 837−847.
(5) Banerji, U.; Judson, I.; Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 385−390.
(6) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260−266.

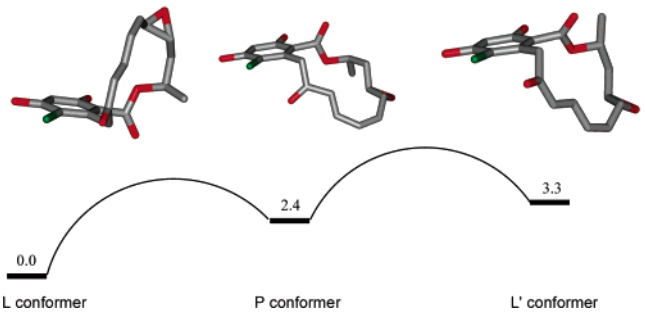


Figure 2. Wire-frame representation of radicicol's three main conformers and their relative energies (kcal).

site.[10−12] Attesting to the potential of HSP90 inhibition for chemotherapy, a derivative of geldanamycin [17AAG (**3**), Figure 1] is currently under clinical evaluation.[13] While radicicol is the most potent inhibitor of HSP90, it lacks activity in vivo presumably due to metabolic instability. It has been shown that thiols such as DTT can inactivate the action of radicicol.[14] This result has been attributed to a 1,6-Michael addition to the conjugate diene yielding an inactive product.[15] Importantly, converting the ketone to an oxime affords products that retain acitivity in vivo by reducing the electrophilicity of the Michael acceptor.[15,16] These compounds were found to be effective against oncogenic mouse xenograft. Additionally, Danishefsky and co-workers have reported a series of analogues where the epoxide has been replaced by a more resistant cyclopropane and showed that these compounds are active in vitro.[17,18] These results have raised the possibility that radicicol can be modified to yield a therapeutic agent. However, in the design of modified inhibitors, one concern has been that many apparently similar compounds have different affinities. Herein, we report a molecular dynamics study that addresses this question by determining the conformational landscape of putative inhibitors (including radicicol) and use the results to design simplified analogues based on conformational similarity to radicicol, which were then synthesized and tested experimentally.

Results and Discussion

Molecular Dynamics Analysis of Radicicol and Potential Analogues. To evaluate the conformation−activity relationship of radicicol, its conformational profile (Figure 2) and that of several reported analogues (Figure 3) were analyzed. Each molecule was simulated by molecular dynamics with the Merck Molecular Force Field (MMFF94)[19−23] in the CHARMM[24] program. A dielectric constant of 80 was used to simulate the effect of solvent in a simple way. The simulations were carried out at 1000 K during 1 ns and 500 frames were extracted from the trajectory at 20 ps intervals. The high temperature was used to ensure that conformational energy barriers were crossed. Each frame was minimized by 750 steps of the steepest descent (SD) algorithm in CHARM, and the MMFF energy was calculated. The resulting 500 conformations were clustered to determine the main conformations. Starting from the lowest energy conformation as representative of the first cluster, all conformations having a root-mean-square deviation (RMSD) lower than 1 Å were grouped into that cluster. The lowest energy conformer of the remaining conformations was taken as the starting point for the second cluster, and the process was repeated until all compounds had been clustered. The RMSD between the lowest energy representative of each cluster and the bioactive conformation of radicicol in the cocrystal structure[6] was then calculated for all heavy atoms. This analysis led to the identification of three main conformations: an L-shape conformation, which is the bioactive conformation of radicicol, an essentially planar (P-shape) conformation, and an L′-shape conformation that mainly differs from the L-shape one by the fact that the macrocycle is positioned on the opposite side of the aromatic cycle (Figure 2). The calculated L-shape, P-shape, and L′-shape conformations of the isolated molecules have an average RMSD from the bioactive radicicol conformation of about 0.6, 1.8, and 2.1 Å, respectively (the bioactive radicicol conformation was taken from the PDB structure ID 1BGQ).[6] Importantly, the

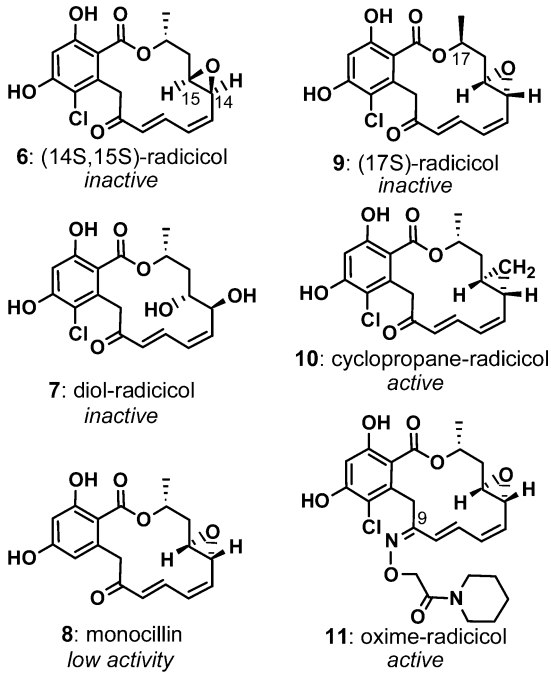


Figure 3. Structures of selected radicicol analogues.

(7) Vilenchik, M.; Solit, D.; Basso, A.; Huezo, H.; Lucas, B.; He, H.; Rosen, N.; Spampinato, C.; Modrich, P.; Chiosis, G. *Chem. Biol.* **2004**, *11*, 787−797.
(8) Chiosis, G.; Lucas, B.; Huezo, H.; Solit, D.; Basso, A.; Rosen, N. *Curr. Cancer Drug Targets* **2003**, *3*, 371−376.
(9) Wright, L.; Barril, X.; Dymock, B.; Sheridan, L.; Surgenor, A.; Beswick, M.; Drysdale, M.; Collier, A.; Massey, A.; Davies, N.; Fink, A.; Fromont, C.; Aherne, W.; Boxall, K.; Sharp, S.; Workman, P.; Hubbard, R. E. *Chem. Biol.* **2004**, *11*, 775−785.
(10) Marcu, M. G.; Chadli, A.; Bouhouche, I.; Catelli, M.; Neckers, L. M. *J. Biol. Chem.* **2000**, *275*, 37181−37186.
(11) Langer, T.; Schlatter, H.; Fasold, H. *Cell Biol. Int.* **2002**, *26*, 653−657.
(12) Yun, B.-G.; Huang, W.; Leach, N.; Hartson, S. D.; Matts, R. L. *Biochemistry* **2004**, *43*, 8217−8229.
(13) http://www.clinicaltrials.gov/ct/gui/show/NCT00004241?order=13.
(14) Kwon, H. J.; Yoshida, M.; Fukui, Y.; Horinouchi, S.; Beppu, T. *Cancer Res.* **1992**, *52*, 6926−6930.
(15) Agatsuma, T.; Ogawa, H.; Akasaka, K.; Asai, A.; Yamashita, Y.; Mizukami, T.; Akinaga, S.; Saitoh, Y. *Bioorg. Med. Chem.* **2002**, *10*, 3445−3454.
(16) Ikuina, Y.; Amishiro, N.; Miyata, M.; Narumi, H.; Ogawa, H.; Akiyama, T.; Shiotsu, Y.; Akinaga, S.; Murakata, C. *J. Med. Chem.* **2003**, *46*, 2534−2541.
(17) Yamamoto, K.; Garbaccio, R. M.; Stachel, S. J.; Solit, D. B.; Chiosis, G.; Rosen, N.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 1280−1284.
(18) Yang, Z.-Q.; Geng, X.; Solit, D.; Pratilas, C. A.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 7881−7889.
(19) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 616−641.
(20) Halgren, T. A.; Nachbar, R. B. *J. Comput. Chem.* **1996**, *17*, 587−615.
(21) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 553−586.
(22) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 520−552.
(23) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519.
(24) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.

Table 1. Relative Energies of Radicicol and Its Derivatives Main Conformers (Energies Are in kcal/mol Relative to the Lowest Conformer Energy)

compound	L-shape	L′-shap e	P-shape	RMSD[a]	simil.[b]
radicicol (**1**)	0.0	3.3	2.4	0.60	0.024
(14*S*,15*S*)radicicol (**6**)	2.7	0.0	1.7	0.60	0.029
diol-radicicol (**7**)	2.7	0.0	1.6	0.61	0.025
monocillin (**8**)	0.0	0.7	2.1	0.56	0.023
(17*S*)-radicicol (**9**)	0.0	3.0	2.3	0.62	0.027
cyclopropane (**10**)	0.0	1.7	1.6	0.56	0.023
oxime (**11**)	0.0	2.1	4.2	0.54	0.025
E-isomer of oxime **11**	0.3	0.0	3.6	0.62	0.026

[a] RMSD between the L-shape conformation and the bio-active conformation of radicicol, calculated for common heavy atoms. [b] Similarity index defined as the RMSD (see footnote a) divided by the number of atoms included in the RMSD calculation; the lower is the similarity index, the greater is the similarity.

bioactive L-shape is the lowest energy conformer found in the molecular dynamics analysis. Its energy is 3.3 and 2.4 kcal/mol lower than that of the L′-shape and P-shape conformers, respectively (Table 1). This result suggested that the wrong conformational bias could be involved in the lack of activity of analogues in which the stereochemistry of specific centers was altered. Indeed, a corresponding conformational analysis of compound **6**[17] in which the epoxide stereochemistry was inverted (Figure 3) showed that, while the macrocycle could be clustered in the same three shapes, the lowest energy conformation was no longer the bioactive conformation. Opening of the epoxide to the corresponding diol (**7**,[25,26] Figure 3) likewise favored the inactive L′ conformation. Removal of the chlorine atom (monocillin, **8**)[27,28] lowers the energetic bias for the bioactive conformation. This result is not surprising considering that this chlorine can contribute to a large allylic 1,3-strain. Furthermore, it was clear from the crystal structure that the chlorine atom could fill a hydrophobic pocket. Interestingly, the stereochemistry at the C17 position (**9**,[17] Figure 3) did not have a significant impact on the conformation. The lack of activity of this compound is likely to be due to its inability to fit in the active site rather than to a change in conformational bias. Compound **10**[17] wherein the epoxide is replaced by a cyclopropane has been shown to have comparable activity to radicicol and indeed has a similar conformational profile. Likewise, compound **11**[16] showed a profile similar to radicicol. The enhanced activity of compound **11** as compared to radicicol can be rationalized by the fact that the oxime substituent fills a hydrophobic pocket (see Figure 4). It is interesting to note that the *E*-isomer of oxime **11** has a less favorable conformational bias for the bioactive L-shape than the *Z*-isomer. This observation is consistent with experimental data from related oximes wherein the *Z*-isomer had a cytotoxicity of 98 versus 282 nM for the *E*-isomer.[18] The above analysis shows that the epoxide function influences the relative stability of the main conformations of radicicol derivatives. In particular, the configuration of the asymmetric carbons of the epoxide function found in radicicol favors the bioactive conformation.

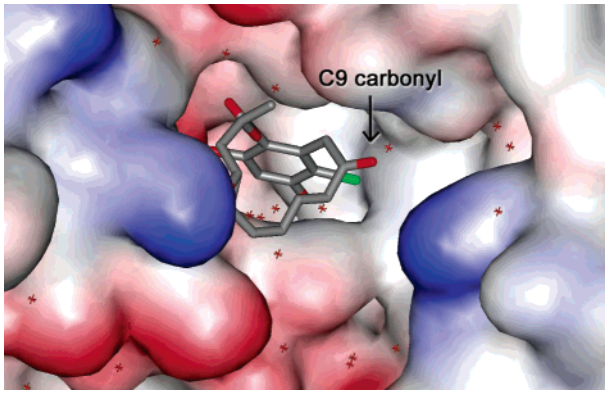


Figure 4. Representation of radicicol (wire-frame structure, see Figure 1 for numbering) bound to HSP90 (represented as a surface, blue indicates positive regions, red indicates negative regions, and + indicates water molecules) [image generated with Weblab from PDB data ID 1BGQ].

Based on the presumed importance of maintaining the bioactive (L-shape) conformation, we profiled the conformation space of a series of compounds with modifications of the epoxide and olefin region (Figure 5). Replacement of the epoxide for an amide bond (**12** and **13**) or a bridging cyclopentane (**15**, Figure 5) led to conformations that disfavored the L-shape. Removal of the epoxide (**17**) led to a compound that strongly disfavors the L-shape. However, replacement of the epoxide by an olefin (**18** and **19**) only moderately disfavored the L-shape conformation. Interestingly, compounds **14** and **16** lacking the α,β-conjugated olefin had an unfavorable bias for the L-shape. Compound **19** caught our attention as it represents a significant simplification in terms of chemical synthesis while only moderately disfavoring the active L-shape conformation (1.2 kcal). Recently, compound **19** was isolated from the fermentation broth of *Pochonia chlamydosporia* and named pochonin D.[29] In the isolation report, the authors did find pochonin D to be cytotoxic at micromolar concentration, which could be rationalized by its HSP90 inhibition. Pochonin D was docked in HSP90 and was found to have a good overlap with radicicol (Figure 6). Given these computational and experimental results, a practical and modular synthesis of pochonin D was sought.

Polymer-Assisted Synthesis of Pochonin D and Analogues Thereof. The synthetic disconnections of pochonin D (**19**, Figure 7) were based on the chemistry that was developed in the context of our total synthesis of pochonin C.[30] We were particularly interested in developing chemistry amenable to using polymer-supported reagents[31−35] for the purpose of diversity oriented synthesis. Commercially available Weinreb amide **23** (Scheme 1) was selectively S-alkylated with 3-hydroxythiophenol and 1 equiv of base, and then loaded onto Merrifield resin in the same pot by adding a second equivalent of K_2CO_3, the

(25) Shibata, T.; Oikawa, T.; Kobayashi, T.; Shimazaki, N. *Jpn. Kokai Tokkyo Koho*; Sankyo Co., Ltd.: Japan, 1997; 5 pp.
(26) Ikeda, A.; Shinonaga, H.; Fujimoto, N.; Kasai, Y. *PCT Int. Appl.*; Taisho Pharmaceutical Co., Ltd.: Japan, 2003; 126 pp.
(27) Ayer, W. A.; Pena-Rodriguez, L. *Phytochemistry* **1987**, *26*, 1353−1355.
(28) Danishefsky, S. J.; Garbaccio, R. M.; Baeschlin, D. K.; Stachel, S. J.; Solit, D.; Shtil, A.; Rosen, N. *PCT Int. Appl.*; Sloan-Kettering Institute for Cancer Research: USA, 2002; 135 pp.
(29) Hellwig, V.; Mayer-Bartschmid, A.; Mueller, H.; Greif, G.; Kleymann, G.; Zitzmann, W.; Tichy, H.-V.; Stadler, M. *J. Nat. Prod.* **2003**, *66*, 829−837.
(30) Barluenga, S.; Lopez, P.; Moulin, E.; Winssinger, N. *Angew. Chem., Int. Ed.* **2004**, *43*, 3467−3470.
(31) Vickerstaffe, E.; Warrington, B. H.; Ladlow, M.; Ley, S. V. *J. Comb. Chem.* **2004**, *6*, 332−339.
(32) Storer, R. I.; Takemoto, T.; Jackson, P. S.; Brown, D. S.; Baxendale, I. R.; Ley, S. V. *Chem.-Eur. J.* **2004**, *10*, 2529−2547.
(33) Bapna, A.; Vickerstaffe, E.; Warrington, B. H.; Ladlow, M.; Fan, T.-P. D.; Ley, S. V. *Org. Biomol. Chem.* **2004**, *2*, 611−620.
(34) Lee, A.-L.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 3957−3966.
(35) Ley, S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815−4195.

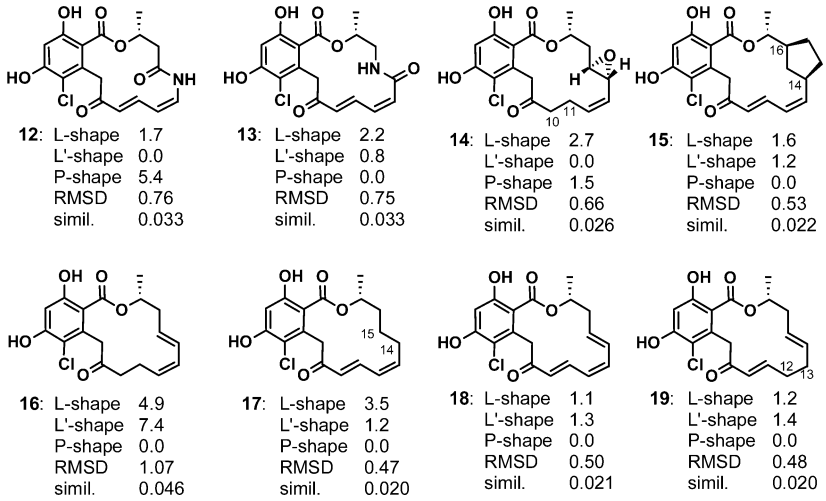


Figure 5. Structure and conformational analysis of designed radicicol analogues.

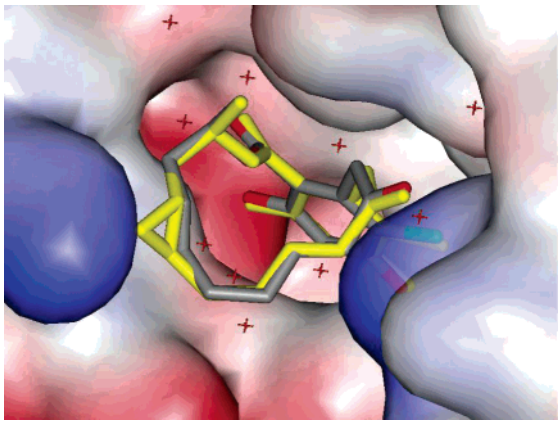


Figure 6. Representation of pochonin D (wire-frame structure) docked in HSP90 superimposed over the structure of radicicol (yellow wire-frame structure); HSP90 is represented as a surface (see Figure 4 for legend) [image generated with Weblab from PDB data].

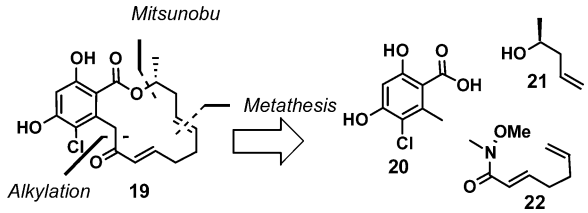
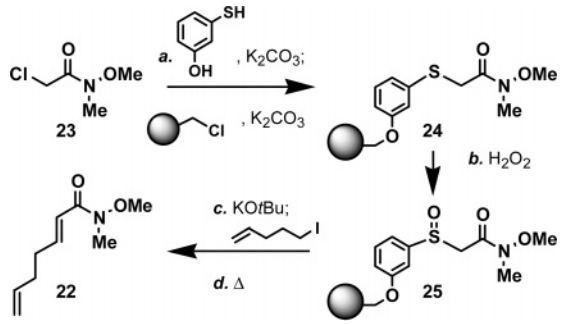


Figure 7. Retrosynthetic disconnections of pochonin D (**19**).

resin, and raising the temperature to 50 °C. While preparations of thiol resins have been reported by direct lithiation of the resin followed by a quench with elemental sulfur,[36] this method was found more practical as it affords the polymer-bound Weinreb amide in one step. Oxidation of the thioether **24** to sulfoxide **25** was carried out using H_2O_2 in HFIP/CH_2Cl_2.[37] This oxidation procedure was found to be practical and reliable with no overoxidation to the sulfone in a number of studied cases. Carrying out the reaction on solid phase also allows for an easy recycling of the fluorinated solvent. The ketosulfoxide **25** was then deprotonated with KO*t*Bu, and the resulting enolate was quenched with 5-iodopentene. The use of DMSO was found to be critical for the success of this reaction; DMF, *t*BuOH, and

Scheme 1. Synthesis of Weinreb Amide Fragment **22**[a]



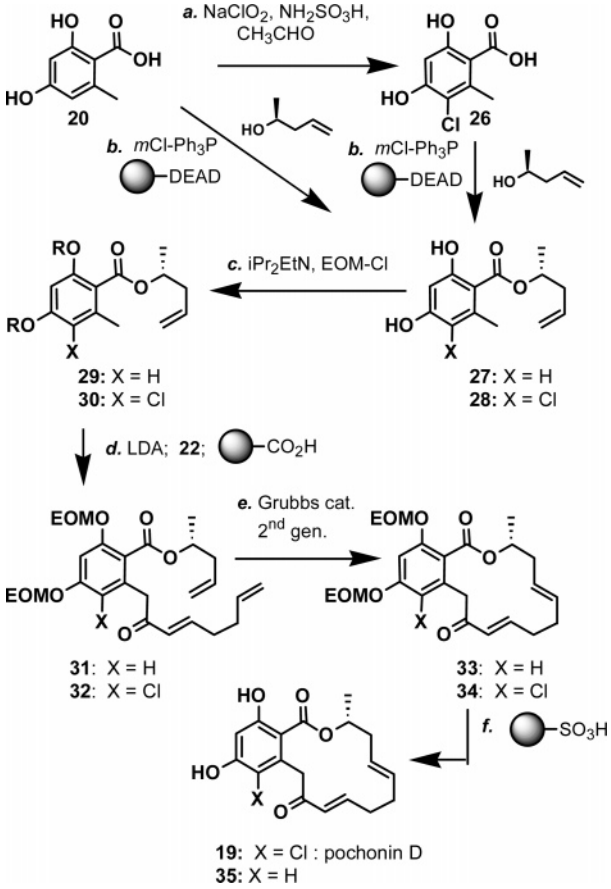
[a] (a) 3-Mercaptophenol (1.0 equiv), K_2CO_3 (1.0 equiv), DMF, 23 °C; after 8 h, K_2CO_3 (1.7 equiv), Merrifield resin, TBAI (cat), 12 h, 50 °C, 98%; (b) H_2O_2 (2.0 equiv), $(CF_3)_2CHOH$ /CH_2Cl_2 (1/1), 12 h; (c) KO*t*Bu (1.0 equiv), 1-iodo-5-pentene (1.0 equiv), DMSO, 23 °C, 3 h; (d) toluene, 80 °C, 8 h, 77%. DMF = *N*,*N*-dimethylformamide, DMSO = dimethyl sulfoxide, TBAI = tetrabutylammonium iodide.

1,4-dioxane gave poor results. Furthermore, the polar nature of DMSO is unfavorable for the sulfoxide elimination, and the reaction could be heated to 60 °C without any elimination which allowed an excess of the alkylating agent to be used. Resuspension of the resin in toluene and heating to 80 °C released the compound by elimination, affording desired fragment **22** with 77% yield and 95% purity (judged by NMR).

Selective Mitsunobu esterification of benzoic acid **20** using polymer-bound DEAD afforded ester **27** (Scheme 2). The use of (*m*ClPh)$_3$P was found to be essential to suppress the competing ether formation with the *para*-phenol.[30,38] Protection of both phenols with EOM−Cl[39] afforded ester **29**, which could be used in the subsequent alkylation without further purification. All attempts to chlorinate ester **27**, **29**, or later intermediates were unsuccessful due to the reactivity of the olefin toward electrophilic chlorine. The chlorine atom was thus introduced prior to esterification of acid **20** using HClO generated in situ by the oxidation of acetaldehyde with $NaClO_2$/sulfamic acid. Esterification of this product under the same conditions as for **20** afforded compound **28**, which was protected with EOM−Cl to obtain ester **30**. As for ester **29**, this product could be used in subsequent reactions without purification. Deprotonation

(36) Farrall, M. J.; Frechet, J. M. J. *J. Org. Chem.* **1976**, *41*, 3877−3882.
(37) Ravikumar, K. S.; Begue, J.-P.; Bonnet-Delpon, D. *Tetrahedron Lett.* **1998**, *39*, 3141−3144.
(38) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487−6491.
(39) EOM-Cl: EOM-Cl was used instead of MOM-Cl for availability reasons.

Scheme 2. Synthesis of Macrocycles **33** and **34**[a]



[a] (a) $NaClO_2$ (5.0 equiv), NH_2SO_3H (5.0 equiv), CH_3CHO (1.0 equiv), THF/H_2O (5/1), 0 °C, 0.5 h, 92%; (b) PS-DEAD (2.5 equiv, 1.3 mmol g^{-1}), *s*-(−)-penten-2-ol (1.0 equiv), P(*m*ClPh)$_3$ (2.0 equiv), CH_2Cl_2, 23 °C, 0.5 h, 68% for **27** and 65% for **28**; (c) $^{i}Pr_2EtN$ (4.0 eqiv), EOMCl (4.0 equiv), TBAI (cat), DMF, 80 °C, 5 h, 95%; (d) LDA (2.0 equiv), THF, −78 °C, **22** (1.0 equiv), 10 min, Amberlite IRC-50 (20.0 equiv, 10.0 mmol g^{-1}); (e) Grubbs II (10% mol), toluene (2 mM), 80 °C, 12 h, 40% after two steps; (f) PS-TsOH (10 equiv, 3.2 mmol g^{-1}), MeOH, 40 °C, 4 h, 90% for **19**; MeOH, 40 °C, 0.5 h, 92% for **35**. PS-DEAD = ethoxycarbonyl-azocarboxymethyl polystyrene, PS-TsOH = sulfonic acid resin MP, DMF = *N*,*N*-dimethylformamide, Grubbs II = ruthenium[1,3-bis-(2,4,6-trimethyl-phenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexyl-phosphine), LDA = lithium diisopropylamide, EOM = ethoxymethylene, TBAI = tetrabutylammonium iodide, THF = tetrahydrofurane.

of the toluic esters **29** and **30** followed by addition of Weinreb amide **22** afforded the desired metathesis precursors **31** and **32** along with 20% undesired product stemming from a 1,4 conjugate addition. Treatment of crude triene **31** and **32** with the second generation Grubbs catalyst[40,41] at 120 °C for 10 min afforded the desired cyclization product **33** and **34** in excellent yield albeit as an unseparable 1:4 mixture of cis:trans. Allowing the reaction to equilibrate under thermodynamic control at 80 °C overnight shifted in both cases the equilibrium to the trans with >95% selectivity.[42] It should be noted that this reaction could be performed at millimolar concentration without any detectable amount of dimerization or oligomerization. Importantly, the 10-membered ring cyclization product was not observed in either case. Moreover, a simple filtration through a short path of silica could be used to remove all of the catalyst and its byproducts to afford compound **34** in 60% yield. While the metathesis reaction carried out on purified triene **32** was nearly quantitative, it was deemed more practical to carry out the whole synthetic sequence from compound **20** without purifications, thus affording the protected pochonin D **34** in 34% yield for five steps. The EOM groups were removed from both macrocycles **34** and **33** using sulfonic acid resin in MeOH to obtain pochonin D (**19**)[43] and its analogue **36** lacking the chlorine in 90% and 92% yield, respectively. It is interesting to note that the conjugated olefin proved to have different reactivity depending on the presence or absence of the chlorine atom on the aryl ring. Deprotection of compound **33** with HCl in dioxane led to the conjugate addition of the chlorine ion, whereas compound **34** proved more resilient to conjugate addition and could be deprotected with HCl to obtain **19**. The difference in reactivity of the conjugate olefins may be attributed to the different conformations of the two compounds, which is due to the 1,3-allylic strain conferred by the bulky chlorine atom. It is conceivable that the differences in conformation affect the level of conjugation between the olefin and the adjacent carbonyl group and thus its susceptibility to Michael addition.

Table 2. Affinity of Radicicol Analogues for HSP90

	IC_{50} (μM)
radicicol (**1**)	0.02
pochonin D (**19**)	0.08
33	>50
34	>50
35	>50

Biological Evaluation of Pochonin D. The compounds were evaluated for HSP90 affinity in a competition assay with geldanamycin using a previously described method.[45] The results are shown in Table 2. Pochonin D (**19**) was found to be a good ligand for HSP90 with an IC_{50} of 80 nM as compared to 20 nM for radicicol. This difference is consistent with the calculated 1.2 kcal change in the internal energy of the pochonin D upon binding. The cocrystal structure of HSP90-radicicol reported by Pearl et al. showed a tightly bound water molecule making a hydrogen-bond bridge between the *ortho*-phenol, the ester, and Asp79 and a second water molecule making a bridge between the *para*-phenol and Leu34.[6] Accordingly, compound **34** with alkylated phenols showed no affinity for HSP90. The importance of the chlorine atom is evident from the comparison of compounds **19** (80 nM) and **35** (>50 μM). The bulky chlorine not only comes within van der Waals contact of Phe124 and fills a hydrophobic void, but it also biases the population of the bioactive conformer due to allylic strain (see relative energies of compound **8** vs **1**, Table 1).

Conclusion and Future Prospects

The conformational analysis of radicicol and its known analogues showed that there are three low-energy conformations and gave a good correlation between their biological activity and the energetic cost of adopting the bioactive L-shape conformation. The importance of the decrease in affinity due

(40) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953−956.
(41) Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 3783−3784.
(42) Lee, C. W.; Grubbs, R. H. *Org. Lett.* **2000**, *2*, 2145−2147.
(43) Synthetic pochonin D was found to have NMR spectra identical to natural pochonin D (spectra were kindly provide by Marc Stadler, Bayer Health Care, Wuppertal, Germany).
(44) Kometani, T.; Kondo, H.; Fujimori, Y. *Synthesis* **1988**, 1005−1007.
(45) Zhou, V.; Han, S.; Brinker, A.; Klock, H.; Caldwell, J.; Gu, X.-j. *Anal. Biochem.* **2004**, *331*, 349−357.

to binding a conformer different from the global minimum in solution has been pointed out previously, although the bioactive conformation of a flexible ligand often does not correspond to the global energy minimum of the free ligand; that is, the binding energy compensates for the reorganization energy and loss of entropy.[46−48] In the present study, the relative conformational energies were evaluated with a simplified solvent representation. Thus, the results are only of qualitative significance. Nevertheless, the conformational analysis provides a rationale for the observed changes in activity due to modifications of the epoxide moiety. Analysis of a series of simplified analogues led to the identification of pochonin D as having a low energy and high similarity index to the bioactive conformation. A polymer-assisted synthesis was developed, which affords pochonin D from readily available fragments **20**, **21**, and **22** in six steps and requires only one chromatographic purification of the final product. This also represents the first total synthesis of pochonin D (**19**), thereby assigning a (+) polarization to (17-*R*)-pochonin D. The affinity of pochonin D for HSP90 (80 nM) is close to that of radicicol (20 nM), the difference being consistent with the energetic cost of adopting the bioactive conformation. As the cyclopropane analogues previously reported,[18,49] the pochonin D scaffold lacks the sensitive epoxide. In a larger context, this study clearly illustrates the dramatic impact of small structural changes on the conformational equilibrium of these resorcyclic macrolides and may rationalize the diverse biological activity of structurally similar compounds from this family.

Acknowledgment. A Marie Curie Fellowship to S.B. (Grant no.: HPMF-CT-2002-01968) and BDI from CNRS and region d'Alsace to E.M. are gratefully acknowledged. We thank Merck Bioscience/Novabiochem for samples of resins, as well as Xiang-ju Gu and Francisco Adrian for their assistance with the biological screening. This work was partly supported by a grant from the European Commission (MIRG-CT-2004-505317) and is part of the COST netword D28. The work done at Harvard was supported by the National Institutes of Health.

Supporting Information Available: Experimental procedures utilized and compound characterization including a NMR of pochonin D. This material is available free of charge via the Internet at http://pubs.acs.org.

JA043101W

(46) Vieth, M.; Hirst, J. D.; Brooks, C. L., III. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 563−572.
(47) Bostrom, J.; Norrby, P.-O.; Liljefors, T. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 383−396.
(48) Perola, E.; Charifson, P. S. *J. Med. Chem.* **2004**, *47*, 2499−2510.
(49) Geng, X.; Yang, Z.-Q.; Danishefsky, S. J. *Synlett* **2004**, 1325−1333.