J|A|C|S
ARTICLES
Published on Web 06/05/2004

New Efficient Synthesis of Resorcinylic Macrolides via Ynolides: Establishment of Cycloproparadicicol as Synthetically Feasible Preclinical Anticancer Agent Based on Hsp90 as the Target

Zhi-Qiang Yang,[†] Xudong Geng,[†] David Solit,[‡] Christine A. Pratilas,[‡] Neal Rosen,[‡] and Samuel J. Danishefsky*[,†,§]

Contribution from the Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021; Program in Cell Biology and Department of Medicine, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021; and Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, New York 10027

Received March 18, 2004; E-mail: s-danishefsky@ski.mskcc.org

Abstract: A program currently ongoing in our laboratory envisions natural macrolide radicicol-based inhibitors targeting the molecular chaperone Hsp90. Such inhibitors can be potential anticancer agents due to their ability to induce the breakdown of a variety of oncogenic proteins. In this account, we first concern ourselves with a vastly important total synthesis of such an inhibitor. We accomplished this via a new approach, which we term the "ynolide method", directed to the synthesis of resorcinylic macrolides, including cycloproparadicicol and aigialomycin D. The key features of the syntheses involve cobalt-complexation-promoted ring-closing metathesis (RCM) to generate ynolides, followed by Diels−Alder reaction with dimedone-derived bis-siloxy dienes to elaborate the benzo system. A number of interesting analogues were synthesized using this protocol. They were evaluated for their inhibitory activity against the growth of breast cancer cell line, MCF-7. The potency of their cytotoxicity was found to be consistent with their ability to degrade the oncogenic protein, Her2. From these assays, cycloproparadicicol was identified as a most promising candidate for further development.

Introduction

The heat shock protein 90 (Hsp90) is a molecular chaperone that mediates the stabilization and folding of various oncogenic proteins, such as *Raf1* and *Her2*.[1] Recently, Hsp90 has attracted extensive attention as a novel, potential antitumor target. The natural product family which has attracted by far the greatest attention as a potential inhibitor of the action of Hsp90 are congeners of geldanamycin (**3**).[2−4] Indeed, a derivative of geldanamycin, 17AAG (**4**), is the most advanced of drug candidates based on Hsp90 and is currently in phase II clinical trials (Figure 1).[1,5]

Though our laboratory has been involved in attempting to build upon 17AAG as a discovery lead centering around Hsp90,[6−8] we hoped to evaluate the potentialities of another natural product targeting Hsp90, i.e., radicicol. We postulated that radicicol-derived drug candidates could in the long run out perform "geldanamycinoids" in that the latter would carry potential liabilities from the ansa bridged quinone substructure. Indeed, in a potentially important comparison, radicicol is significantly less hepatotoxic than 17AAG.[9] However, radicicol was found to be ineffective in vivo animal models. We theorized

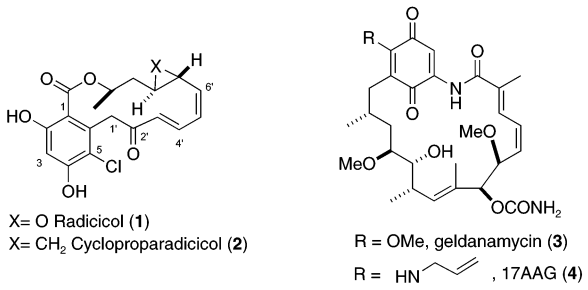
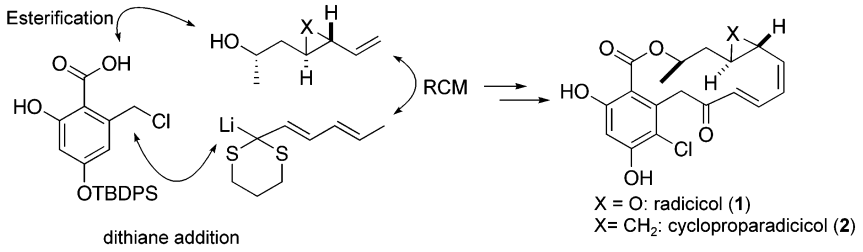


Figure 1. Structures of Hsp90 inhibitors.

[†] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research.
[‡] Program in Cell Biology and Department of Medicine, Sloan-Kettering Institute for Cancer Research.
[§] Department of Chemistry, Columbia University.

(1) Banerji, U.; Judson, I.; Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 385−390.
(2) Delmotte, P.; Delmotte-Plaquee, J. *Nature* **1953**, *171*, 344.
(3) Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, Y. *Can. J. Microbiol.* **1980**, *26*, 766−773.
(4) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260−266.
(5) DeBoer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* **1970**, *23*, 442−447.
(6) Kuduk, S. D.; Harris, C. R.; Zheng, F. F.; Sepp-Lorenzino, L.; Ouerfelli, O.; Rosen, N.; Danishefsky, S. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1303−1306.
(7) Zheng, F. F.; Kuduk, S. D.; Chiosis, G.; Munster, P. N.; Sepp-Lorenzino, L.; Danishefsky, S. J.; Rosen, N. *Cancer Res.* **2000**, *60*, 2090−2094.
(8) Kuduk, S. D.; Zheng, F. F.; Sepp-Lorenzino, L.; Rosen, N.; Danishefsky, S. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1233−1238.
(9) Chiosis, G.; Lucas, B.; Huezo, H.; Solit, D.; Basso, A.; Rosen, N. *Curr. Cancer Drug Targets* **2003**, *3*, 371−376.

Scheme 1. Our First Total Synthesis of Cyclopropradicicol



that the epoxide both in radicicol and in radicicol oxime (which is active in in vivo models[10]) could well be a source of nonspecific cytotoxicity, which could narrow the exploitable margin of therapeutic index. Furthermore, the potential chemical vulnerability of the dienyl epoxide raised concerns about drug shelf stability as well as pharmacokinetics. With a view to molecular editing, of the oxido function in a setting of minimal conformational perturbation of the radicicol lead, we were drawn to analogue **2** in which the epoxide linkage is replaced by a cyclopropane.

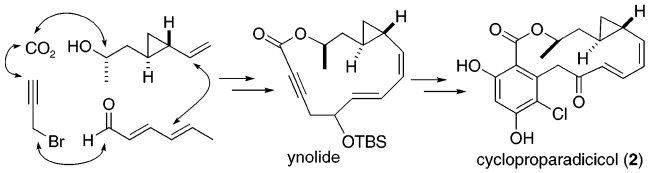
We had previously shown that compound **2** has an in vitro biological profile comparable to that of **1**.[11] Moreover, we brought to bear an important line of evidence that **2** and **1** were closely related in their interactions with their biotargets. Thus changes in peripheral stereogenic centers in both **1** and **2** bring about the same consequences in biological function. In other words, both structures as shown are optimized from a stereochemical perspective. Moreover, and of considerable potential advantage for radicicol-based inhibitors, they also display cytotoxicity against Rb (retinoblastoma)-negative cells known to be resistant to 17AAG.[11]

These promising findings inevitably raised issues as to the availability of cyclopropradicicol. Clearly, our first synthesis of this compound,[11] while touching on several issues of academic interest in organic chemistry, did not seem promising for producing more than token amounts of the now interesting **2**.

As seen, the first synthesis from our lab relied on the appropriate sequenced Mitsunobu esterification, dithiane alkylation, and ring closing olefin metathesis. While highly convergent and concise, this first generation pathway to radicicol suffered from several low yielding steps which did not improve following attempted optimization. In particular, the low yields associated with the dithiane alkylation and ring-closing metathesis (RCM) steps sharply curtailed access to cyclopropradicicol for evaluation.[11]

Indeed, a new strategy has been formulated and reduced to practice, resulting in a much improved second generation total synthesis of cyclopropradicicol.[12] In addition, the new route shows promise of applicability to a broad range of resorcinylic macrolides.[13] Herein, we disclose the full details of this new approach and its application to reach cyclopropradicicol as well as aigalomycin D. The synthesis and biological activities of a number of interesting analogues of cyclopropradicicol will be described. Based on the much improved route of synthesis, and

Scheme 2. New Ynolide Approach for Cyclopropradicicol



based on early investigations of biological profiles, cyclopropradicicol now emerges as a candidate for full scale preclinical evaluation.

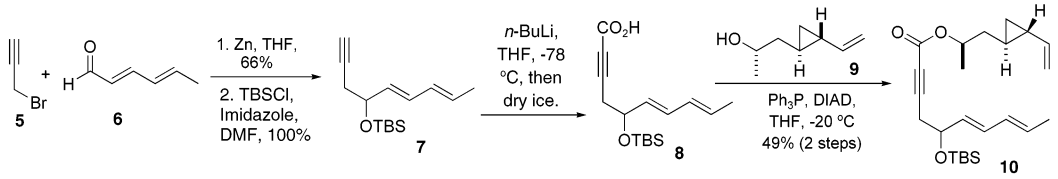
Results and Discussions

Overall Strategy. The defining element of our second generation strategy was the building of the aromatic sector of the resorcinylic marcrolide by Diels−Alder reaction of a new type of dienophile, i.e., an "ynolide". This cycloaddition route to the benzo-fused macrolactone represents a substantial departure from the usual mode of synthesis in which one starts with an aromatic ring and appends to it suitable arms to close the macrolactone ring (cf. Schemes 1 and 2). We hoped that the new ynolide approach, if successful, would be highly convergent and would allow for rapid access to a broad family of resorcinylic macrolides. Since it has been our experience that monoactivated acetylenic dienophiles are surprisingly weakly reactive in Diels−Alder reactions,[14] the success of a projected Diels−Alder cycloaddition aromatization sequence could not have been anticipated with confidence.

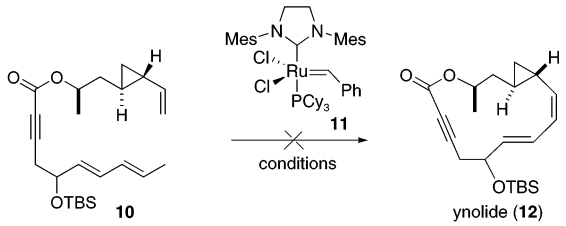
Synthesis of Acyclic Alkynoic Ester. To facilitate progress to the key issues of the plan, we sought to develop an efficient synthesis route to reach a seco-alkynoate ester, which, following RCM, would afford the ynolide dienophile. In practice, the synthesis commenced with commercial 2,4-hexadienal (sorbaldehyde, **6**, Scheme 3). Reformatsky-like condensation of propargyl bromide (**5**) with **6** led to the expected carbinol. Following β-esterification, alkyne precursor **7** was in hand in good yield.[15,16] Treatment of **7** with *n*-butyllithium generated an alkynide ion, which was carboxylated with dry ice, to provide acid **8**, in very high yield.[17] Following reaction of racemic **8** and the known optically pure and defined alcohol **9**[18] under Mitsunobu conditions,[19] ester **10** was obtained as a diastereomeric mixture at the future $C_{2'}$.

(10) Agatsuma, T.; Ogawa, H.; Akasaka, K.; Asai, A.; Yamashita, Y.; Mizukami, T.; Akinaga, S.; Saitoh, Y. *Bioorg. Med. Chem.* **2002**, *10*, 3445−3454.
(11) 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.
(12) Yang, Z.-Q.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 9602−9603.
(13) Geng, X.; Danishefsky, S. J. *Org. Lett.* **2004**, *6*, 413−416.
(14) Danishefsky, S.; Etheredge, S. J. *J. Org. Chem.* **1979**, *44*, 4716−4717.
(15) Friedrich, L. E.; De Vera, N.; Hamilton, M. *Synth. Commun.* **1980**, *10*, 637−643.
(16) Barrett, A. G. M.; Pena, M.; Willardsen, J. A. *J. Org. Chem.* **1996**, *61*, 1082−1100.
(17) Fuganti, C.; Pedrocchi-Fantoni, G.; Sarra, A.; Servi, S. *Tetrahedron: Asymmetry* **1994**, *5*, 1135−1138.
(18) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10903−10908.
(19) Romo, D.; Rzasa, R. M.; Shea, H. A.; Park, K.; Langenhan, J. M.; Sun, L.; Akhiezer, A.; Liu, J. O. *J. Am. Chem. Soc.* **1998**, *120*, 12237−12254.

Scheme 3. Synthesis of the Acyclic Alkynoic Ester



Scheme 4. Attempted Ring-Closing Metathesis Reactions of **10**



entry	Conditions	results
1	0.25 equiv **11**, CH_2Cl_2, 40 °C, 0.5 mM, 2 h	recovered **10**
2	0.25 equiv **11**, toluene, 120 °C, 0.5 mM, 1 h	recovered **10**
3	1.1 equiv **11**, toluene, 120 °C, 0.2 mM, 10 min	**10** decomposed
4	0.3 equiv **11**, 0.3 equiv Ti(O*i*-Pr)$_4$, CH_2Cl_2, 0.5 mM, 40 °C, 2 h	recovered **10**

Macrocyclization via Ring Closing Olefin Metathesis. The next key reaction was the formation of our hoped macrocyclic ynolide through ring-closing olefin metathesis. For this purpose, we made recourse to the powerful metalo carbene catalysts, innovated by Grubbs and associates, based on ligand **11**.[20] However, as shown in Scheme 4, all efforts to form the desired ynolide **12** using catalytic amounts of **11** provided the recovered starting ester **10**. The Lewis acid, Ti(O*i*-Pr)$_4$, was used in the hope of preventing formation of an inactive complexing locus which included the ester group. However, this attempt at reaction steering was also not successful. Attempted increase of the amounts of **11** to stoicheometric levels eventually led to decomposition of **10**.

The failure of probe structure **10** to cyclize could be ascribed to conformational rigidities associated with the trans-disubstituted cyclopropane and the linear acetylene "linker". Accordingly, a more flexible model ester **14** was prepared (in 59% yield) using standard protocols. Compound **14** was subjected to RCM conditions (Scheme 5). Unfortunately, no formation of cyclic product was observed.

Cobalt Complexation to Promote RCM. At this stage, we reasoned that the failure of olefin metathesis might be due to the presence of the acetylene group. Aside from the constraint to cyclization imposed by its linear character, the cyclization could further be complicated by nonproductive coordination of the acetylene to the RCM catalytic machinery. The latter possibility could not be dismissed out of hand, since the enyne metathesis reactions are well established.[21−23] Accordingly, protection of the alkyne could well be helpful. It is well-known that reactions of dicobalt carbonyl with acetylenes can lead to stable complexes, in which the alkyne functions are, in effect, protected.[24,25] Moreover, the geometry of cobalt-complexed alkynes optimizes at approximately 140°.[26] Such a departure from linearity could well favor cyclization.[27] We thus hoped that cobalt protection of the alkyne could serve to our advantage in the problematic macrocyclization.

To probe the feasibility of this hypothesis, model ester **14** was first treated with cobalt dicarbonyl (Scheme 6). Complex formation proceeded readily at room temperature in toluene to provide **15** (in 87% yield) after silica gel chromatography. When complex **15** was subjected to the conditions of ring closing olefin metathesis, with catalyst, cyclization proceeded smoothly at room temperature. The cobalt-protected ynolide **16** was obtained in 71% using 25 mol % of **11** and high dilution (0.2 mM) in methylene chloride.

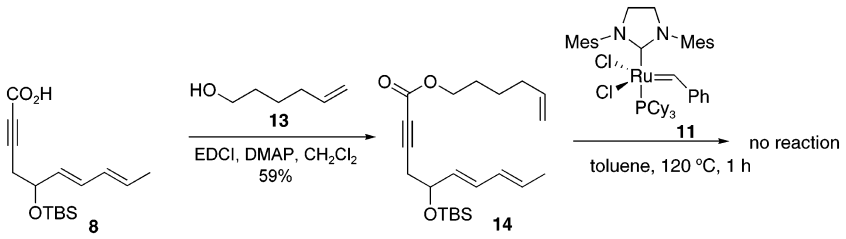
To retrieve the free acetylene of the ynolide, the cobalt complex had to be "demetalated." This goal was readily achieved by treatment of **16** with excess ceric ammonium nitrate in acetone at low temperature.[28] Ynolide **17** was obtained in excellent yield (Scheme 6).

We next applied this strategy to the target system **10**. Gratifyingly, as was the case with the model compound **15**, RCM reaction of cobalt-complexed **18** proceeded smoothly at room temperature in CH_2Cl_2 to afford cyclic product **19** in 57% yield. Postmetathesis removal of the cobalt, however, was nontrivial. Treatment of **19** with CAN in acetone led primarily to decomposition. Other oxidizing agents such as I_2[29] or Me_3NO[30] improved the yields significantly but were not fully reproducible when conducted in large scales. Eventually, it was found that when the solution of **19** was buffered with 2,6-di-*tert*-butyl pyridine prior to the treatment with CAN, the desired product **12** was isolated in 50% yield in two steps from **18** (Scheme 7).[31] At this stage, the two stereoisomers of **12** became separable, and they were advanced individually.

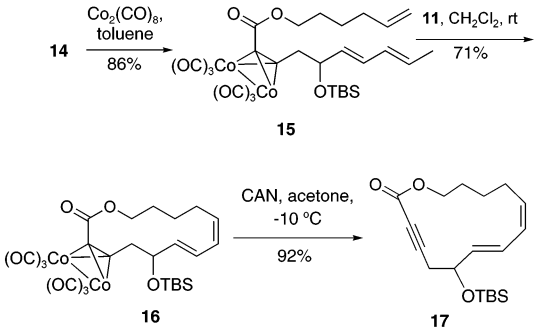
Cycloaddition−Aromatization Reactions. Having achieved an efficient synthesis of the required ynolide, we directed our attentions to the next challenge, i.e., the fashioning of the desired resorcinylic macrolides using a Diels−Alder elimination sequence. We appreciated that acetylenic dienophiles, where only one of the sp carbons is activated with a typical activating group such as an ester can be rather unreactive.[14] These monoactivated acetylenes must be clearly distinguished at the planning level

(20) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953−956.
(21) Kinoshita, A.; Mori, M. *Synlett* **1994**, 1020−1022.
(22) Kinoshita, A.; Mori, M. *J. Org. Chem.* **1996**, *61*, 8356−8357.
(23) Mori, M.; Sakakibara, N.; Kinoshita, A. *J. Org. Chem.* **1998**, *63*, 6082−6083.
(24) Greenfield, H.; Sternberg, H. W.; Friedel, R. A.; Wotiz, J. H.; Markby, R.; Wender, I. *J. Am. Chem. Soc.* **1956**, *78*, 120−124.
(25) Nicholas, K. M.; Pettit, R. *Tetrahedron Lett.* **1971**, *37*, 3475−3478.
(26) Dickson, R. S.; Fraser, P. J. *Adv. Organomet. Chem.* **1974**, *12*, 323−377.
(27) Recently, a similar protocol was reported: Young, D. G. J.; Burlison, J. A.; Peters, U. *J. Org. Chem.* **2003**, *68*, 3494−3497.
(28) Magnus, P. *Tetrahedron* **1994**, *50*, 1397−1418.
(29) Tanaka, S.; Tsukiyama, T.; Isobe, M. *Tetrahedron Lett.* **1993**, *34*, 5757−5760.
(30) Jones, G. B.; Wright, J. M.; Rush, T. M.; Plourde, G. W., II.; Kelton, T. F.; Mathews, J. E.; Huber, R. S.; Davidson, J. P. *J. Org. Chem.* **1997**, *62*, 9379−9381.
(31) Magnus, P.; Eisenbeis, S. A.; Fairhurst, R. A.; Iliadis, T.; Magnus, N. A.; Parry, D. *J. Am. Chem. Soc.* **1997**, *119*, 5591−5605.

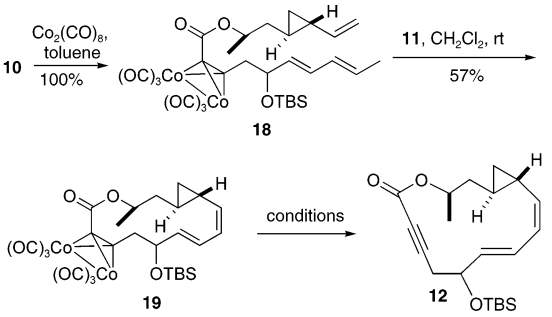
Scheme 5. Olefin Metathesis Reaction of Model Ester **14**



Scheme 6. RCM of Cobalt-Protected Model Ester

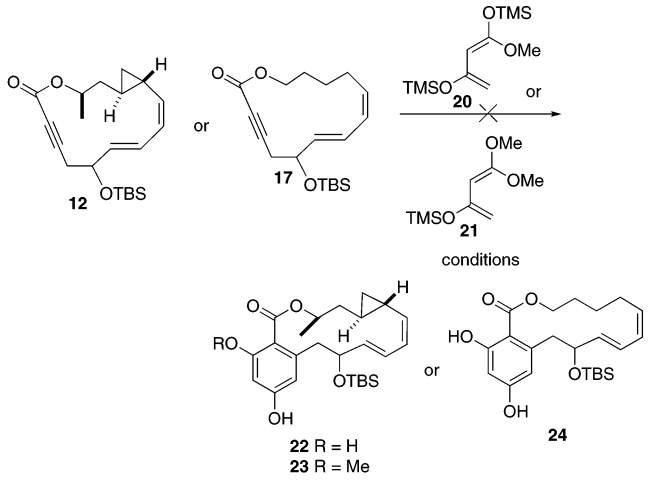


Scheme 7. Synthesis of Ynolide **12**



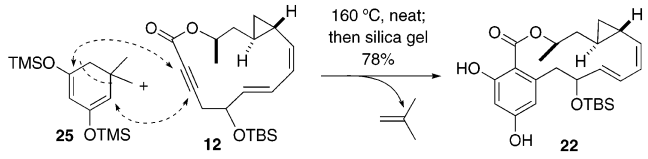
Entry	conditions	yields
1	CAN, acetone, -10 °C	<10%
2	I_2, THF, 0 °C	≤ 69%
3	Me_3NO, acetone/THF -78 °C to rt	66%
4	CAN, DTBP, acetone, -10 °C	50% from **18**

Scheme 8. Attempted Diels−Alder Reactions with Acyclic Dienes



Entry	Conditions	Results
1	**12**, **20**, neat, 75 °C; then $Et_3N{\cdot}HF$, EtOH	recovered desilylated **12**
2	**12**, **21**, neat, 160 °C; then 0.1 N HCl	Decomposition
3	**17**, **20**, EuFOD, neat, 70 °C; then $Et_3N{\cdot}HF$, EtOH	recovered desilylated **17**
4	**17**, **20**, Ti(O*i*-Pr)$_4$, neat, 70 °C	recovered **17**

Scheme 9. Diels-Alder Reaction of **12** with Cyclic Diene **25**



from diactivated acetylenes such as diesters of acetylenedicarboxylic acid which are powerful dienophiles.

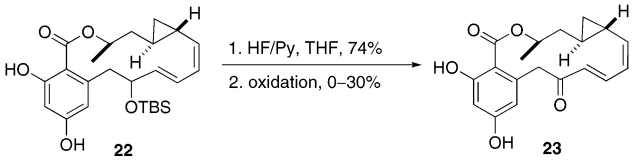
At first, trioxygen-substituted dienes of the type **20**[32] and **21**[14,33,34] seemed to be appropriate choices to attempt cycloaddition with ynolides **12**. Ideally, reaction of **12** with diene **20** followed by elimination of MeOH would, upon desilylation, lead to the desired free phenol product **22**. The use of diene **21** would require an additional step to deprotect the phenolic methyl ether of the product **23** (Scheme 8). Such deprotections had been found to be nontrivial in related systems.[18]

To our surprise, when **12** and diene **20** were heated to 70 °C, no aromatic products were isolated. Desilylated **12** was recovered. Since **20** is prone to undergo a 1,5-silyl shift at temperatures above 70 °C,[26] we directed our efforts to the thermally more stable diene **21**. Unfortunately, even when **12** and diene **21** were heated to 160 °C, no products were isolated. The ynolide **12** had decomposed. In the hope of lowering the temperature required for the cycloaddition, we turned to the use of Lewis acids. Considering the extreme acidic liability of these dienes, we chose mild reagents Eufod[35−37] in the context of projected Diels−Alder reactions of model ynolide **17** with diene **21**. Unfortunately, no product formation was observed.

We next directed our attentions to the use of the dimedone-derived cyclic diene **25**[38,39] (Scheme 9). The latter could, in principle, react with ynolide diastereomers **12** to afford desired product **22** after elimination of isobutylene.[40,41] The possible advantages of this diene over tri-oxygen-substituted acyclic possibilities, such as **20** or **21**, are that the cyclic diene could

(32) Yamamoto, K.; Suzuki, S.; Tsuji, J. *Chem. Lett.* **1978**, 649−652.
(33) Banville, J.; Brassard, P. *J. Chem. Soc., Perkin Trans. I* **1976**, 1852−1856.
(34) Danishefsky, S.; Singh, R. K.; Gammill, R. B. *J. Org. Chem.* **1978**, *43*, 379−380.
(35) Danishefsky, S.; Harvey, D. F.; Quallich, G.; Uang, B. J. *J. Org. Chem.* **1984**, *49*, 392−393.
(36) Castellino, S.; Sims, J. J. *Tetrahedron Lett.* **1984**, *25*, 2307−2310.
(37) Lopez, R.; Carretero, J. C. *Tetrahedron: Asymmetry* **1991**, *2*, 93−96.
(38) Ibuka, T.; Mori, Y.; Aoyama, T.; Inubushi, Y. *Chem. Pharm. Bull.* **1978**, *26*, 456−465.
(39) Langer, P.; Schneider, T.; Stoll, M. *Chem.−Eur. J.* **2000**, *6*, 3204−3214.

Scheme 10. Oxidation in the Presence of Free Phenols



entry	Oxidation conditions	Yields
1	MnO_2,	$\leq$ 30%
2	TFAA/DMSO/Et_3N, CH_2Cl_2	Decomposition
3	PCC, NaOAc, CH_2Cl_2	Decomposition
4	Dess-Martin periodinane, CH_2Cl_2	decomposition
5	TPAP, CH_2Cl_2, MS 4Å	decomposition

well be more reactive (due to a locking in of the *s*-syn conformation by the six-member ring) and more thermally stable. Happily, when diene **25** and dienophile **12** were heated to 160 °C, followed by desilylation, in the course of silica gel chromatography, the desired product, **22**, was obtained in 78% yield. Thus a new, highly efficient and convergent method to assemble the resorcinylic macrolide **22**, needed for cyclopropradicicol, had been achieved.

Completion of the Synthesis. To complete the synthesis of cyclopropradicicol (**2**), two remaining steps were required. Oxidation of the 2° alcohols to the corresponding ketone and regioselectve introduction of a chlorine had to be accomplished in some as yet unspecified order. We first attempted to carry out the oxidation of the secondary homobenzylic alcohol in the presence of the free phenolic hydroxyls (Scheme 10). Following the removal of the TBS ether group (using HF/pyridine) in 74% yield, we surveyed a variety of oxidation conditions. Unfortunately, in the best case, we obtained only 30% yield through the use of activated MnO_2.[42,43] The use of other oxidizing agents examined resulted in the decomposition of starting material, **22**.[44−48]

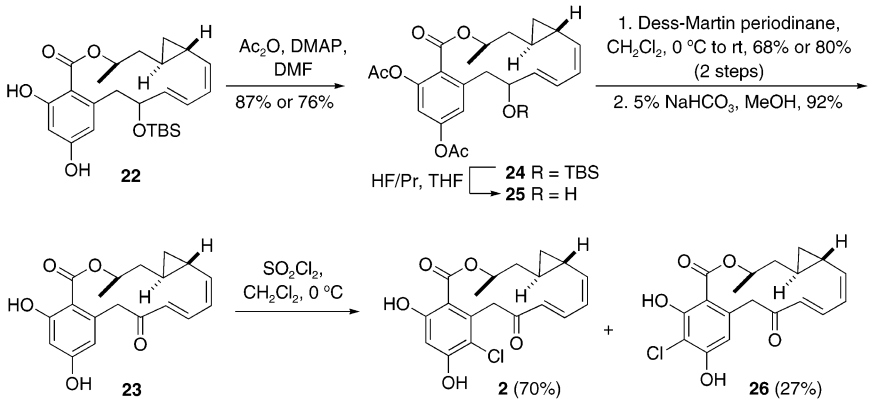
Accordingly, we were obliged to protect the two free phenolic hydroxyl groups. Treatment of **22** with acetic anhydride in DMF and cat. amount of *N*,*N*-dimethylamino pyridine (DMAP) provided diacetate **24** (87% from the major isomer of **22** and 76% from the minor isomer of **22**) (Scheme 11). Subsequent removal of its TBS ether gave alcohol **25**. This deprotection was followed by oxidation using Dess−Martin periodinane, to afford the desired ketone intermediate in 68% yield from the major isomer of **25** (80% yield from the minor isomer). The stereochemistry of two isomers of **25** could be determined by a modified Mosher's analyses (see Supporting Information for details).[49] The acetate groups were cleaved under mildly basic conditions (5% $NaHCO_3$/MeOH, 1:1) in excellent yield.[18] Finally, regioselective chlorination provided cyclopropradicicol (**2**) in 70% yield. Unfortunately, this product was accompanied by the formation of an isomeric chlorination product **26** in 27% yield.[18] Although the issues governing such chlorinations have not necessarily been optimized, this level of regioselectivity seems to be quite general for such resorcinylic compounds.[50,51] In summary, we have developed a new concise synthesis for cyclopropradicicol, in 6% yield following 13 steps.

Synthesis of Analogues and Formulation of a Preliminary SAR Pattern in the Cyclopropradicicol Series. The new synthesis developed here not only allowed us to generate sufficient quantities of cyclopropradicicol to support our biological studies on the compound itself but also allowed us to fashion a series of interesting analogues to study structure−activity relationships. To evaluate the biological impact of modification of the ketone group, two alcohol isomers **27**α,*β* were prepared (Scheme 12). Using SO_2Cl_2, the chloride derivative **28**α was synthesized in one step from the major isomer, **27**α. The methyl ether derivative of **27**α, **29**α was also prepared under neutral conditions (MeI/Ag_2O).[52] Oxime analogues of cyclopropradicicol [(*Z*)- and (*E*)-**30**] were synthesized as well.[10]

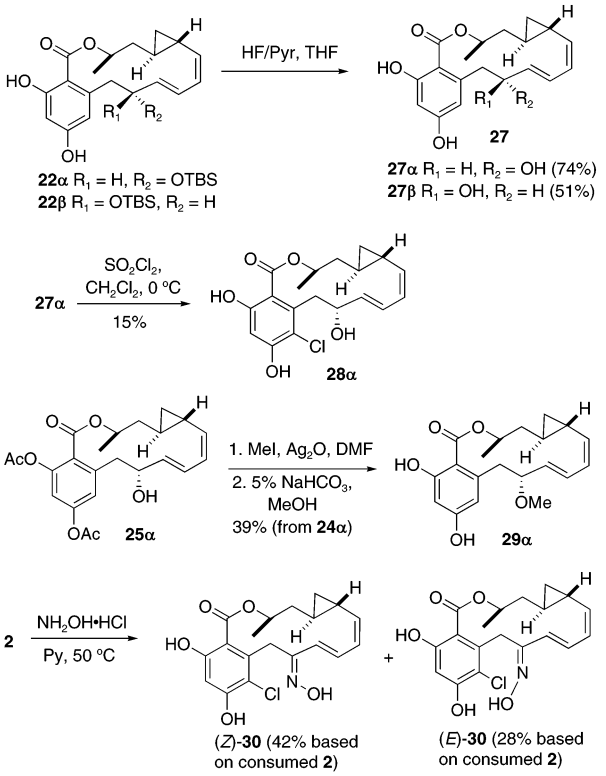
Synthesis of Difulorocyclopropradicicol. Our laboratory recently discovered that a trifluoro-derivative of epothilone exhibits a far superior in vivo profile.[53] Accordingly, we sought to synthesize difluorocyclopropradicicol **31**, hoping to identify even better druglike molecules targeting Hsp90.

The difluorocyclopropane group was introduced to the known conjugated ester **32**, using reagents developed by Dolbier and co-workers, thereby affording difluoroesters **33** as a 1:1 mixture of stereoisomers (Scheme 13).[54] Our goal here was to achieve the target rapidly using the methodology already described in this account. Hence, the mixture of stereoisomers was carried forward through the required synthetic steps. As outlined in Scheme 13, the key ynolide **36** was prepared in reasonable yields following the now familiar protocols described above. The subsequent Diels−Alder reaction with diene **25**, however, proved to be very difficult, affording only 18% of product. Increasing the reaction temperature further eroded the yield, presumably due to the thermal instability of the difluorocyclopropane-containing ynolide **35**. Nevertheless, using the post-

Scheme 11. Completion of the Synthesis of **2**



Scheme 12. Synthesis of Cycloproparadicicol Analogues



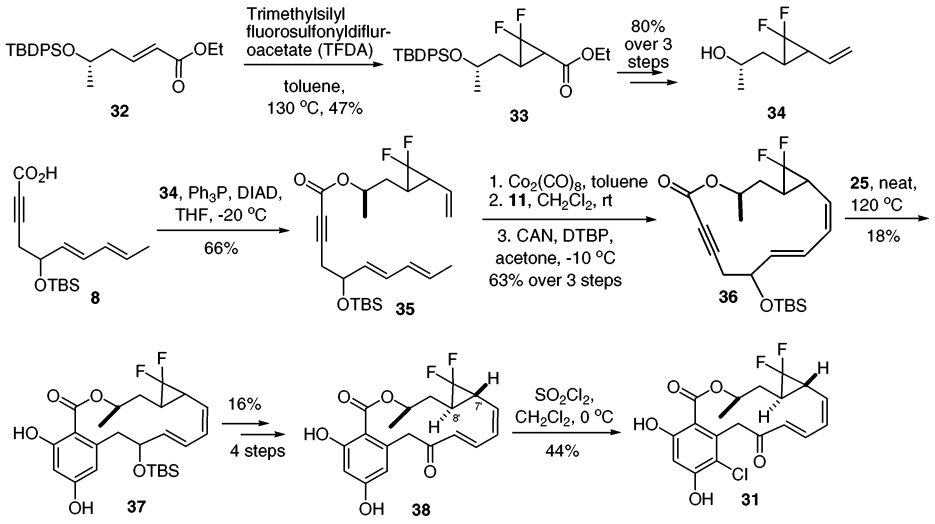
Diels−Alder sequence described previously, adequate amounts of **31** and **38** were obtained to support in vitro studies. It will be noted that, after Dess−Martin oxidation, ketone **38** was isolated as a single isomer. Seemingly, this difluoroycloproplyl isomer of **38** appears to have the same cyclopropane centers (7′R, 8′R) as does cycloproparadicicol itself, based on ^{1}H NMR and optical rotation comparisons (see Supporting Information for details). Presumably, the alternate trans cyclopropyl diastereomer fares poorly in terms of yield in going through the steps from **35** to the end.

Synthetic Approach to the Cycloproparadicicol Lactam. One of the structural features of radicicol that might have contributed to its instability in vivo, in addition to the 7′-8′ epoxide, is the macrolactone. The latter could be subject to metabolic hydrolysis by esterases. With this in mind, we also set out to synthesize the lactam version of the cycloproparadicicol (see compound **39**).

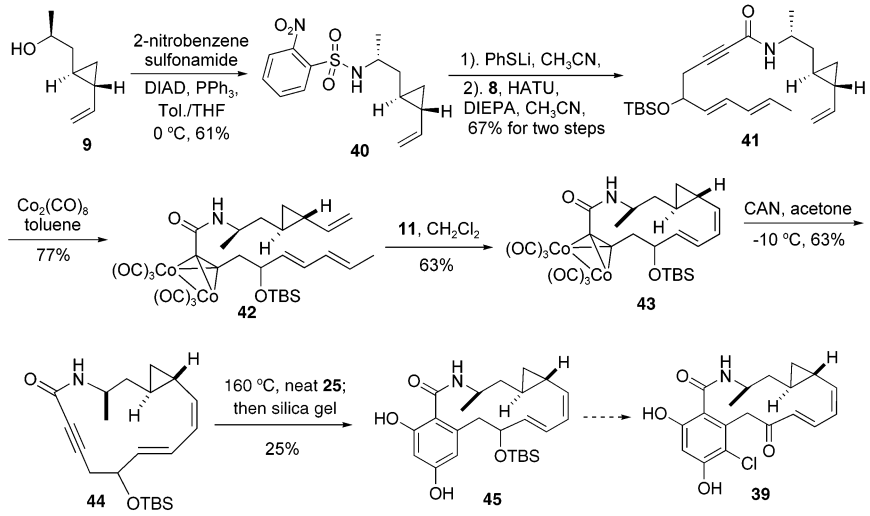
To introduce the functionality required to reach **39**, a sequence involving Staudinger reduction of an azide was considered.[55] However, Mitsunobu type methodology,[56] including Thompson's protocol,[57] failed to deliver the corresponding azide of compound **9**. This is probably due to the instability of the azide compound. Fortunately, it was found that the Nosyl approach recently developed by Fukuyama[58] afforded the desired sulfonamide **40** in 61% yield (Scheme 14). After the removal of the sulfonyl group, the resulting amine, generated in situ, coupled with alkynoic acid **8** to afford amide **41**. Application of the protocol described above (cobalt complexation → RCM → oxidative decomplexation) to the case at hand furnished "ynelactam" **44**. The Diels−Alder reaction of **44** with diene **25**, however, proved to be highly problematic. The best yield realized for reaching product **45** was only around 25%, and that proved to be difficultly reproducible. It is likely that replacement of the ester group by an amide function attenuates the electronegative pull on the acetylene, thereby eroding the reactivity of this linkage as a dienophile. Currently, investigations of other types of more reactive dienes are actively underway in our laboratory to deal with the problem of poorly reactive acetylenic dienophiles. Success on this front would allow us to operate more effectively with weakly reactive dienophiles such as **44**. Pending solution of the synthetic problem, studies directed to the synthesis of **39** have been deferred.

Extension of the Ynolide Approach to the First Total Synthesis of Aigialomycin D. Nature has provided a variety of biologically significant natural products which share the same structural motif as radicicol, i.e., a 14-member resorcinylic macrolide. We sought to test the scope and limitations of our newly developed ynolide-DA approach to such ring systems by applying it to an interesting member of this family, agialomycin D (**46**) (Figure 2). Recently, this compound was isolated from the marine mangrove fungus *Aigialus parvus* BCC5311.[59] It exhibited potent antimalarial activity (IC_{50}: 6.6 μg/mL against *P. falciparum*) and antitumor activity (IC_{50}: 3.0 μg/mL against KB cells).[59] We saw agialomycin D as an attractive candidate expanding upon our new "ynolide" strategy. An important issue to be overcome would be the proper emplacement of the E 1′, 2′ double bond requested to reach

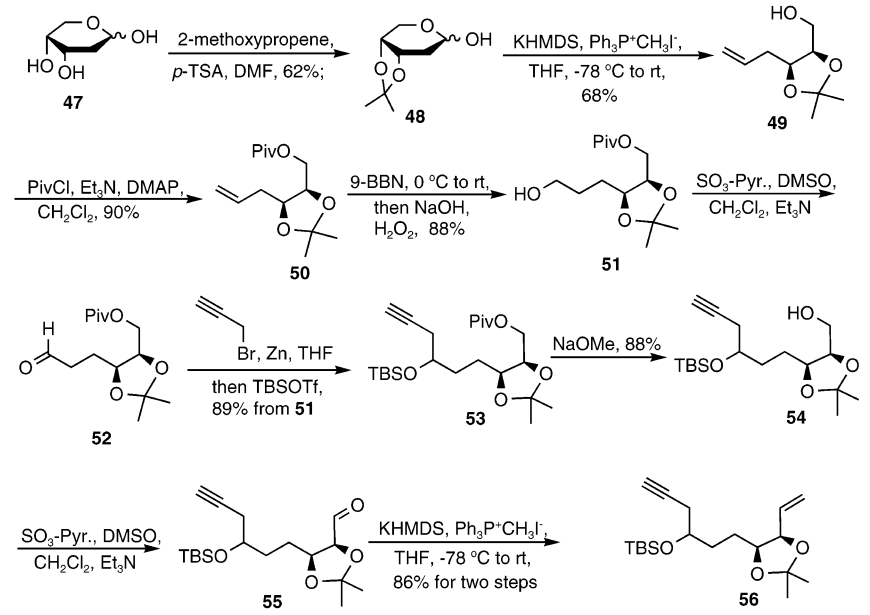
Scheme 13. Synthesis of Difluorocyclopropradicicol



Scheme 14. Synthesis of Cyclopropparadicicol Lactam



Scheme 15. Synthesis of Alkyne **56**



aigialomycin D. Also, the route must accommodate the hydroxy bearing stereogenic centers at carbons 5′ and 6′.

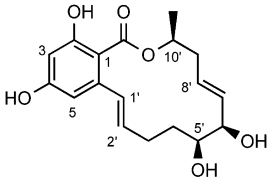


Figure 2. Structure of aigialomycin D.

As shown in Scheme 15, the total synthesis commenced from the naturally derived D-2-deoxy-ribose **47**, which already has the desired future syn 5′,6′-diol functionality of aigialomycin D in place. Compound **47** was processed forward via diol protection, Wittig olefination, hydroboration, and oxidation, to furnish key aldehyde **52**, as shown in Scheme 15. This compound was subjected to nucleophilic propargylation followed by protection of the resultant alcohol to afford TBS ether **53**. The pivaloyl group of **53** was then removed, and a vinyl

(40) Uchiyama, M.; Kimura, Y.; Ohta, A. *Tetrahedron Lett.* **2000**, *41*, 10013−10017.
(41) Morrison, C. F.; Burnell, D. J. *Tetrahedron Lett.* **2001**, *42*, 7367−7369.
(42) Gritter, R. J.; Wallace, T. J. *J. Org. Chem.* **1959**, *24*, 1051−1056.
(43) Trost, B. M.; Caldwell, C. G.; Murayama, E.; Heissler, D. *J. Org. Chem.* **1983**, *48*, 3252−3265.
(44) Huang, S. L.; Omura, K.; Swern, D. *J. Org. Chem.* **1976**, *41*, 3329−3231.
(45) Hauser, F. M.; Prasanna, S.; Combs, D. W. *J. Org. Chem.* **1983**, *48*, 1328−1333.
(46) Welch, S. C.; Levine, J. A.; Arimilli, M. N. *Synth. Commun.* **1993**, *23*, 131−134.
(47) Tatsuta, K.; Takano, S.; Sato, T.; Nakano, S. *Chem. Lett.* **2001**, 172−173.
(48) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639−666.
(49) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296−1298.
(50) Elix, J. A.; Barclay, C. E.; Lumbsch, H. T.; Wardlaw, J. H. *Aust. J. Chem.* **1997**, *50*, 971−975.
(51) Elix, J. A.; Crook, C. E.; Hui, J.; Zhu, Z. N. *Aust. J. Chem.* **1992**, *45*, 845−855.
(52) Tanis, S. P.; Robinson, E. D.; McMills, M. C.; Watt, W. *J. Am. Chem. Soc.* **1992**, *114*, 8349−8362.
(53) Chou, T.-C.; Dong, H.; Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Tong, W. P.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 4762−4767.
(54) Tian, F.; Kruger, V.; Bautista, O.; Duan, J.-X.; Li, A.-R.; Dolbier, W. R., Jr.; Chen, Q.-Y. *Org. Lett.* **2000**, *2*, 563−564.

Scheme 16. Completion of the Synthesis of Aigialomycin D

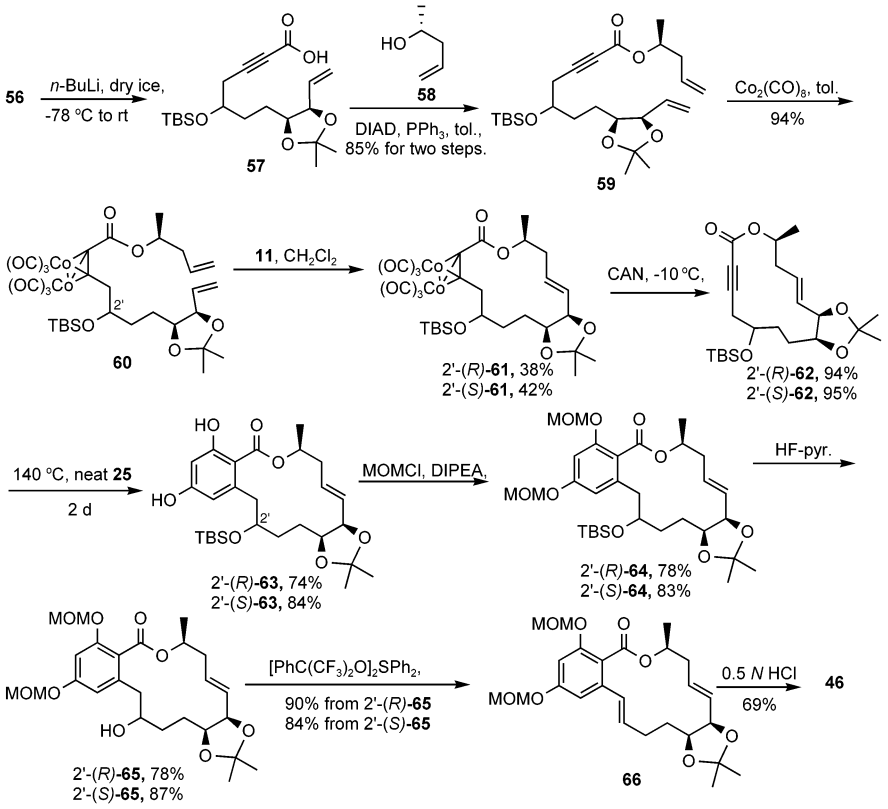


Table 1. IC_{50} (nM) Values of Cycloproparadicicol Analogues and Aigialomycin D

compound	**2**	**26**	**27α**	**27β**	**28α**	**29α**	(*Z*)-**30**	(*E*)-**30**	**31**	**38**	**46**
IC_{50}	54	>500	150	>500	390	>10 000	98	282	10 000	3000	>10 000

group was installed through oxidation and Wittig reactions (see compound **56**).

Following chemistry of the type described above, compound **56** was advanced to ester **58** through carboxylation of the alkyne function and Mitsunobu esterification using the commercially available (*2R*)-4-penten-2-ol. We again investigated the possibility of closure on **58**, itself containing the free alkyne moiety as attempted above (see **10** and **14**). Again, starting material (**58**) was recovered following attempted RCM. Fortunately, we found that our newly discovered protocol (cobalt complexation followed by RCM and then decomplexation) proceeded very efficiently delivering ynolide **61**.

The two benzylic stereoisomers of **61** (2′-(*R*) and 2′-(*S*)) were separated by silica gel chromatography, and their absolute configurations were determined by modified Mosher's ester methodology.[49] As now expected, Diels−Alder reaction between **62** and diene **25** proceeded with high efficiency to afford the desired macrolide **63** in good yield. Following the protection of the phenol hydroxyl groups as the corresponding MOM ethers, the 2′-silyl group was removed and the dehydration reaction was carried out using the Martin sulfurane reagent.[60] Finally, global deprotection under acidic conditions completed the total synthesis of aigialomycin D **46**. The physical data (^{1}H and ^{13}C NMR, Mass spectrum, optical rotation, and IR)[59] in the context of the synthetic sequence and earlier spectral data serve to establish, independently, the structure of synthetic agialomycin D as shown. Moreover, the data for the final synthetic material are fully consistent with those reported for the natural product.[59]

The accomplishment of the total synthesis of aigialomycin D serves to suggest the generalizability of our newly developed protocol. We are hoping to utilize this enhanced methodology to discover resorcinylic derivatives, with significant biological activity.

Biological Evaluation of Cycloproparadicicol Analogues. As discussed earlier, it appears that the inhibition of Hsp90 induces the proteasomal degradation of various oncogenic proteins, thereby leading to inhibition of tumor cell growth. We hoped to evaluate our synthetic analogues for their growth inhibition activity against MCF breast cancer cells. The IC_{50} values of our synthetic compounds were determined after treatment of the cells with each drug listed in Table 1 for 72 h. Cycloproparadicicol is the most potent of the new compounds. Interestingly, chloride regioisomer **26** was found to be inactive. Of the two alcohol isomers, **27α** displayed an IC_{50} value of

(55) Stachel, S. J.; Lee, C. B.; Spassova, M.; Chappell, M. D.; Bornmann, W. G.; Danishefsky, S. J.; Chou, T. C.; Guan, Y. *J. Org. Chem.* **2001**, *66*, 4369−4378.
(56) Mitsunobu, O. *Synthesis* **1981**, 1−28.
(57) Thompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 5886−5888.
(58) Kurosawa, W.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 8112−8113.
(59) Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561−1566.
(60) Martin, J. C.; Arhart, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 4327−4329.

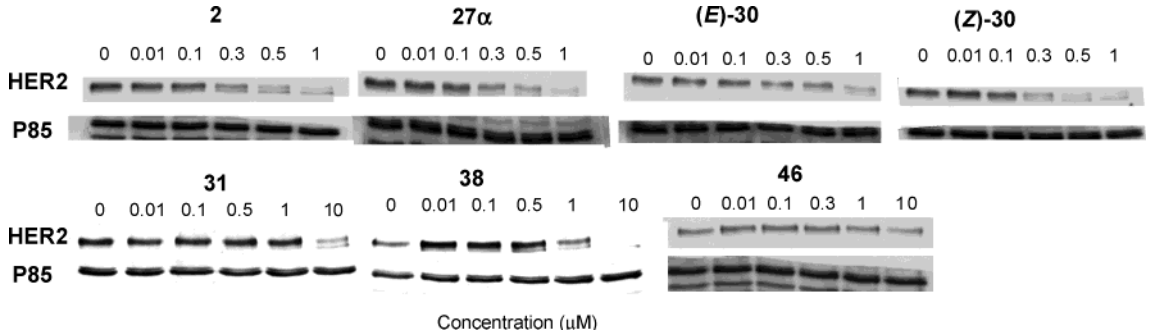


Figure 3. Her2 degradation assay.

150 nM, and **27**β is inactive. Further modifications of **27**α, however, decreased its potency. Surprisingly, introduction of the chlorine function (**28**α) resulted in raising the IC_{50} to 390 nM. Compound **29a**, methyl ether derivative of **27**α, is completely inactive. The (*Z*)-oxime, (*Z*)-**30**, was found 3 times more potent that the corresponding (*E*)-isomer. Aigialomycin D was significantly less active than the cycloproparadicicol derivatives.

To confirm that the cytotoxicity was indeed due to the inhibition of Hsp90, we set out to investigate the degradation of Her2, one of the most sensitive client proteins of this chaperone. The expression levels of Her2 of drug-treated MCF-7 cells were analyzed using immunoblotting (Figure 3). P85, used as a control, is not a client protein of Hsp90. As expected, the growth inhibition activities of the synthetic active analogues are reflected in their capacity to degrade Her2. The three most active compounds, **2**, **27**α, and (*Z*)-**30**, all were able to degrade Her2 at 0.3 μM. (*E*)-**30** is less effective. Nonchlorinated difluoro compound **38** degraded Her2 at 1 μM, and its chlorinated counterpart **31**, at 10 μM. Aigialomycin D (**46**) did not degrade the protein even at 10 μM.

Conclusions and Future Directions

In summary, we have developed a new and efficient synthesis enabling the full evaluation of cycloproparadicicol as a feasible candidate for further advancement. The highlights of the new synthesis are cobalt-complexation-promoted RCM to generate ynolides, followed by Diels−Alder reaction with dimedone-derived bis-siloxyl diene to assemble the benzofused macrolides. Given this pathway, we can generate gram quantities of cyloproparadicicol for biological evaluations. The generality of the synthesis plan has been demonstrated by its application to the first total synthesis of aigialomycin D.

It is, however, appropriate to point out that the sluggish dienophilicity of monoactivated acetylenes is still a problem preventing full generalizability of the method. For instance, in the work described above, i.e., in the cases of the yne lactam **44** and the ynolide **36** containing the sensitive difluorcyclopropyl group, the yields of the Diels−Alder step are low.

The biological activities of our synthetic analogues shown here, in conjunction with the earlier demonstration that cycloproparadicicol binds to Hsp90 at ca. 160 nM,[11] support the notion of the latter as the likely target for this new group of anticancer agents. In vivo evaluations of cycloproparadicicol are in progress in various settings and will be described upon completion of full preclinical studies.

Acknowledgment. This work was supported by the National Institutes of Health (CA-28824). Z.Q.Y. gratefully acknowledges a postdoctoral fellowship from Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Cancer Foundation for Research and The Experimental Therapeutics Center of Memorial Sloan-Kettering Cancer Center. The U.S. Army Breast Cancer Foundation postdoctoral fellowship support is gratefully acknowledged by X.G. (BC022120). We thank Ms. Anna Dudkina and Ms. Sylvi Rusli (NMR Core Facility, CA-02848) for mass spectral analyses, Dr. George Sukenic for NMR analyses, and Dr. William Berkowitz for helpful discussions.

Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0484348