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Design, Synthesis, and Structure−Activity Relationships for Chimeric Inhibitors of Hsp90

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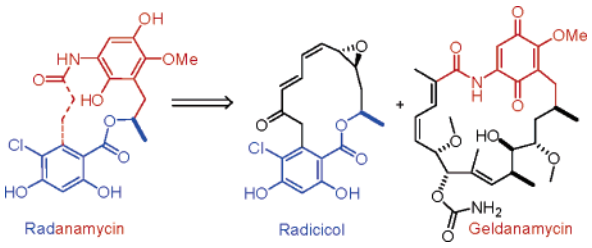

Inhibition of the 90 kDa heat shock protein (Hsp90) family of molecular chaperones represents a promising new chemotherapeutic approach toward the treatment of several cancers. Previous studies have demonstrated that the natural products, radicicol and geldanamycin, are potent inhibitors of the Hsp90 N-terminal ATP binding site. The cocrystal structures of these molecules bound to Hsp90 have been determined, and through molecular modeling and superimposition of these ligands, hybrids of radicicol and geldanamycin have been designed. A series of macrocylic chimeras of radicicol and geldanamycin and the corresponding *seco*-agents have been prepared and evaluated for both antiproliferative activity and their ability to induce Hsp90-dependent client protein degradation.

Introduction

The 90 kDa heat shock proteins (Hsp90) represent a promising target for the development of cancer chemotherapeutics as a consequence of their ability to fold nascent oncogenic polypeptides into functional proteins.[1−3] Not surprisingly, the Hsp90 protein-folding process requires ATP as the requisite source of energy for this conformational maturation process. However, unlike most ATP-binding proteins, Hsp90 binds ATP in a bent conformation that is highly unusual.[4,5] Only eukaryotic enzymes, MutL,[6] and histidine kinase[7] have been shown to bind nucleotides in a similar manner. Moreover, two natural products have been shown to be potent inhibitors of the Hsp90-mediated protein-folding process, and there are currently more than 20 clinical trials in progress based on Hsp90-targeted drugs.[1] All of these trials utilize derivatives of geldanamycin (GDA, Figure 1[8]), which has been shown to produce toxicity unrelated to Hsp90 inhibition. In contrast, the most potent Hsp90 inhibitor identified, radicicol (RDC), exhibits no activity in vivo, as it is rapidly metabolized into inactive compounds that have little or no affinity for Hsp90.[9]

To circumvent problems associated with the natural product inhibitors of Hsp90, several institutions have developed small molecule inhibitors of the Hsp90 molecular chaperone that are based on the purine skeleton,[10,11] dihydroxyphenyl pyrazoles,[12] sulfonamides,[13] or novobiocin.[14] Representative examples of these inhibitors are shown in Figure 2 along with their reported IC_{50}

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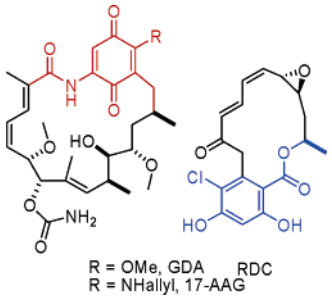

FIGURE 1. Chemical structures of geldanamycin and radicicol.

values in cancer cells.[14] As can be seen, these compounds have not demonstrated similar activity to either geldanamycin or radicicol. However, many of these scaffolds were only recently identified and are still undergoing modification to enhance inhibitory activity.

Although these new inhibitors may prove useful for the treatment of cancer via Hsp90 inhibition, structure−activity relationships for the natural products have not been fully investigated. The first and only total synthesis of geldanamycin was reported in 2003 by Andrus and co-workers,[15] which led to the natural product in slightly more than 40 steps. Problems resulting from a terminal oxidation step proved especially deleterious. Consequently, a new approach has been developed as an alternative method for formation of the GDA quinone.[16] In contrast, Danishefsky and colleagues have reported the total synthesis of radicicol via a [4+2] Diels−Alder reaction between a diene and a propargylic ester to afford both the natural product and a number of analogues.[17,18]

In an effort to develop new Hsp90 inhibitors and to elucidate structure−activity relationships for these natural products, we superimposed the cocrystal structures of GDA and RDC bound to Hsp90.[5,19] As can be seen in Figure 3, the resorcinol moiety of RDC projects into the same binding region as the carbamate of GDA and the purine ring of ADP (Figure 4). In contrast, the quinone ring of GDA extends into the protein−solution interface in analogy to the macrocyclic ring and the epoxide of RDC. Although the entire three-dimensional crystal structure of Hsp90 has not been solved, experimental data and individual structures of the N- and middle domains have supported contact between these two regions, suggesting the region that binds to the quinone and epoxide is not solvent exposed.[20−22] To increase interactions of each inhibitor with Hsp90, we proposed that a molecule containing portions of each natural product should be capable of producing effective Hsp90 inhibitors that could be easily modified for increased potency.

The previously reported cocrystal structures of GDA and RDC bound to yeast Hsp90[5] showed the quinone ring of GDA and

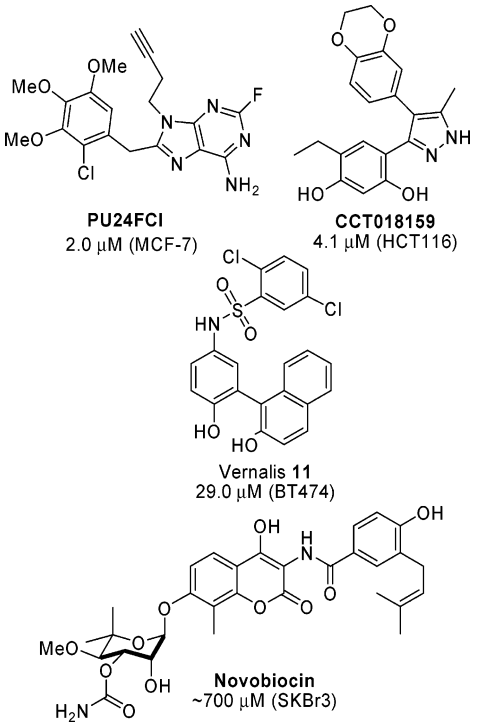

FIGURE 2. Structures of Hsp90 inhibitors and their IC_{50} values in cancer cell lines.

the resorcinol moiety of RDC to bind in opposite orientations and provide key interactions that are responsible for their inhibitory activities. Figure 4 illustrates that four hydrogen bonds are formed between the resorcinol ring and amino acids Leu34, Asp79, Gly83, and Thr171 through conserved water molecules. The carbamate of GDA provides similar interactions with these amino acids, while the quinone ring in GDA affords five additional hydrogen-bonding interactions with amino acids at the surface of the protein.[5] Based on this information, we proposed that inhibitors containing the resorcinol moiety of radicicol and the quinone ring of geldanamycin would provide a starting point for the development of improved analogues by virtue of the additive binding interactions afforded by these individual structures with Hsp90. The chimera of RDC and GDA that is linked by the ester functionality has been named radester, whereas linkage through the amide named radamide and the macrocyclic product appropriately called radanamycin[8,23] (Figure 5).

Although molecular modeling can provide a conceptual starting point for the development of new inhibitors, structure−activity relationships must also be established to further delineate the proposed mode of binding. Therefore, we chose to modify the radester scaffold at several locations in an effort to experimentally probe the basis of Hsp90 inhibition for this series of compounds. The quinone ring is important for GDA's inhibitory activity, however, preliminary results demonstrated the hydroquinone species to be more active in both antiproliferation and client protein degradation assays.[8,24] Recently, this was also observed for 17-AAG, which entered phase I clinical trials as the hydroquinone species.[25] We believe this observed

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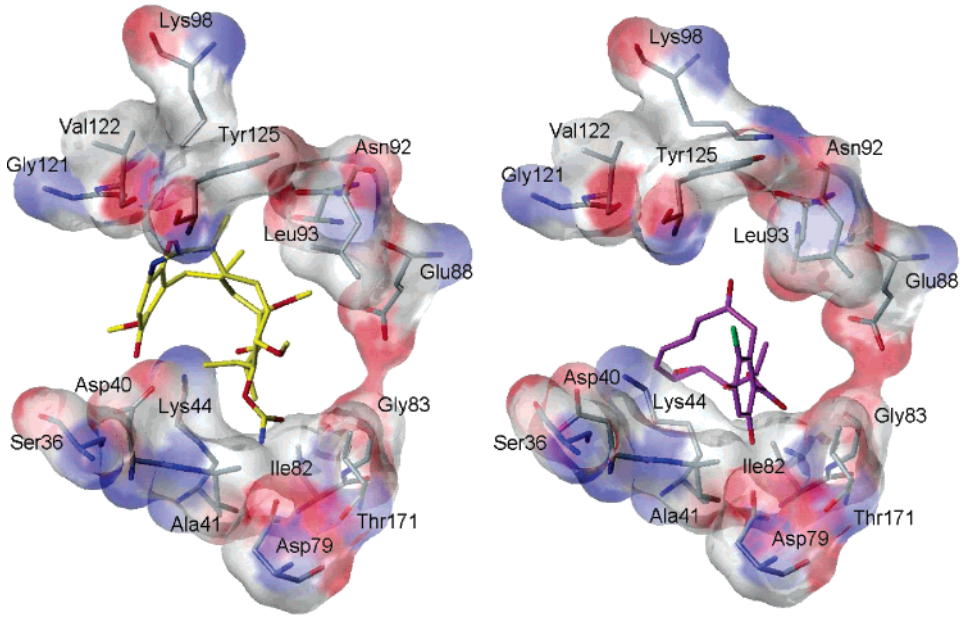

FIGURE 3. Conformations adopted by GDA (yellow) and RDC (violet) in the Hsp90 ATP binding pocket.

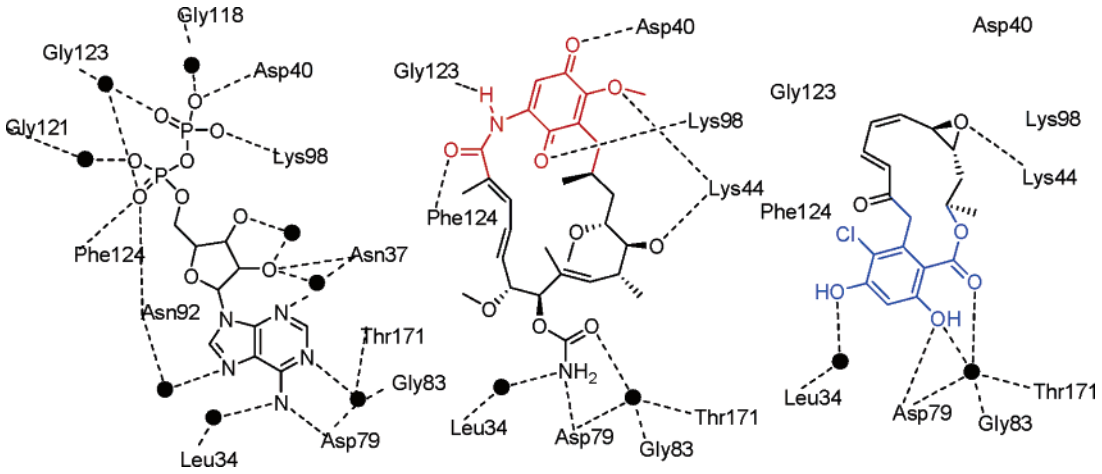

FIGURE 4. Key interactions of ADP, GDA, and RDC with the Hsp90 N-terminal ATP binding pocket.

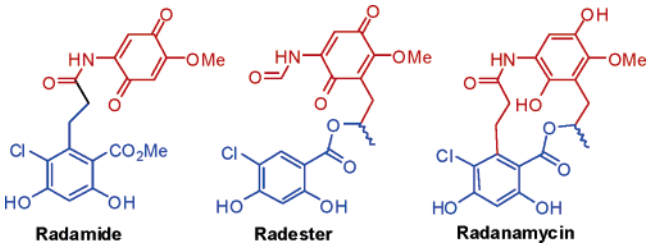

FIGURE 5. Chimeric inhibitors of Hsp90.

activity stems from a complementary binding interaction with Asp40, which in the case of the quinone results in repulsive interactions. The ester is a potential liability as it may be a substrate for lipases. Therefore, the corresponding amide was also prepared for direct comparison (Figure 6). Because both GDA and RDC have branched alkyl groups in the proximity of Phe138, both primary and secondary esters of radester were synthesized. The chlorine substituent on the resorcinol ring projects into a large hydrophobic pocket that is occupied by derivatives of the purine-based Hsp90 inhibitors,[10] suggesting that incorporation of larger hydrophobics may lead to increased affinity. Finally, the length of the tether that connects the quinone and resorcinol rings was experimentally optimized.

To complement our previous studies with radester and radamide, we proposed that conformationally constrained macrocyclic analogues of our hybridized compounds would be more potent primarily from an entropic standpoint.[23] That is, the macrocyclic compounds would be unable to adopt as many conformations as the seco-agents and, thus, would pay a smaller entropic penalty upon binding to Hsp90. In addition, it is suspected that the conformationally predisposed compounds would be more selective for Hsp90 because they would not be able to bind other protein targets that require a different conformation that can be readily accommodated by the seco-agents. In this article, we report the synthesis and evaluation of radester and radanamycin along with the preparation of several analogues and their inhibitory activities.

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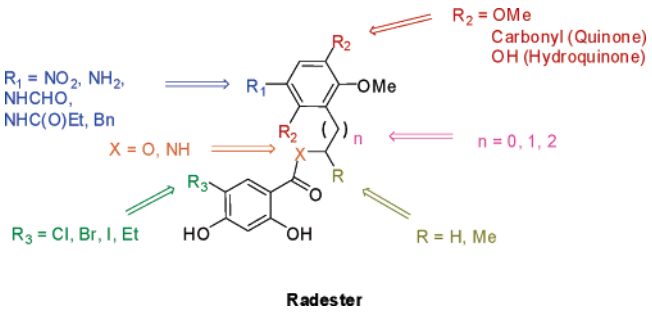


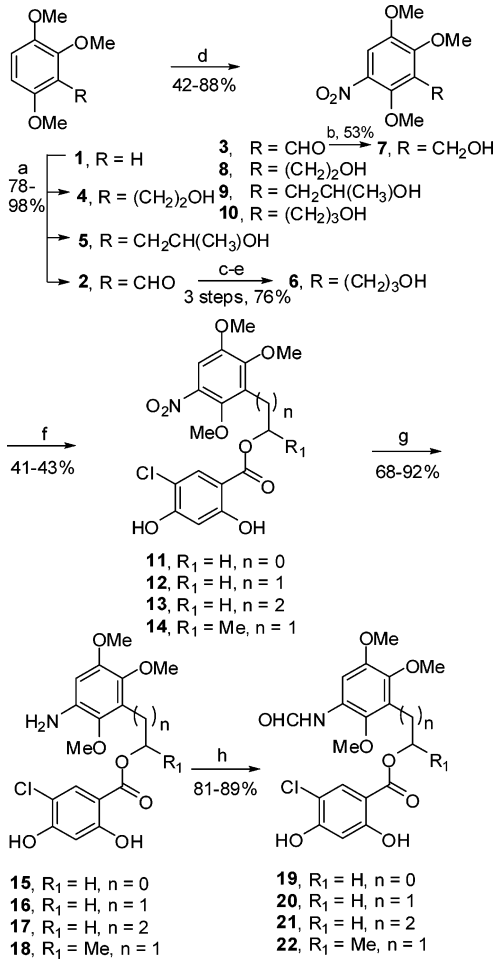
FIGURE 6. Modification of radester skeleton for elucidation of SAR.

Results and Discussion

Radester and Derivatives. Trimethoxy derivatives of the radester quinone ring represented the first series of compounds to be investigated because such a moiety would provide nonredox-active analogues of the quinone and should decrease deleterious side effects of the corresponding moiety found in GDA. Commercially available trimethoxybenzene was treated with *n*-butyllithium to generate the requisite orthoanion, which was reacted with various electrophiles to furnish the corresponding alcohols and aldehyde (**4**, **5**, and **2**). Alcohols **3** and **6** were generated from aldehyde **2** by reduction and homologation followed by reduction, respectively.[15,26] The resulting aromatic rings were nitrated under standard conditions of acetic acid and 70% nitric acid to provide compounds **7**−**10**.[27] Coupling of these alcohols with 2,4-dihydroxy-5-chlorobenzoic acid in the presence of dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine gave esters **11**−**14**. Reduction of the nitro subtituents with hydrogen on platinum oxide afforded anilines **15**−**18**.[28] In the case of GDA, there is an amide functionality at this location, therefore, we converted the aniline species to the corresponding formamide with phenylformate to furnish compounds **19**−**22**[29] (Scheme 1). Attempts to convert the trimethoxy compounds to the corresponding quinones provided little if any of the desired products, as shown in Table 1.

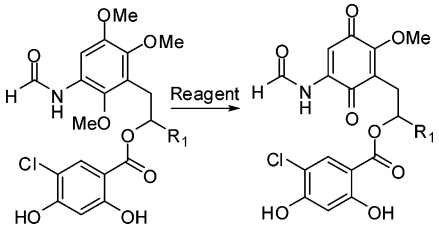
To access the desired quinones, we chose to follow precedence established by Andrus and co-workers, who protected the *para*-hydroxyl groups as the corresponding MOM ethers. Treatment of 2-methoxyhydroquinone with sodium hydride and methoxymethyl chloride gave the MOM ethers, which were then alkylated to provide compounds **26**−**30**.[15,26] Nitration of the acid-labile aromatic rings required mild conditions such as trifluoroacetic anhydride and ammonium nitrate to afford compounds **31**−**35**.[38] Coupling of these alcohols with 2,4-dihydroxy-5-chlorobenzoic acid, followed by reduction of the

SCHEME 1. Synthesis of Trimethoxy Radester[a]



[a] Conditions: (a) *n*-BuLi, E+; (b) HOAc, 70% HNO_3; $NaBH_4$; (c) $(EtO)_2P(O)CH_2CO_2Et$; (d) H_2, Pd/C; (e) *i*-Bu_2AlH; (f) DCC, DMAP, 2,4-dihydroxy-5-chlorobenzoic acid, 50 °C or DIAD, PPh_3; (g) H_2, PtO_2; (h) PhOCHO.

TABLE 1. Oxidation of Trimethoxy Radester to Quinone



reagents	yields
MnO_2[30]	trace
CAN[31]	no reaction
CAN/2,6-pyridine dicarboxylic acid[32]	trace
BBr_3, then O_2[33]	trace
HNO_3/HOAc[34]	3%
DDQ[35]	trace
AgO[36]	10%
10 M H_2SO_4[37]	no reaction

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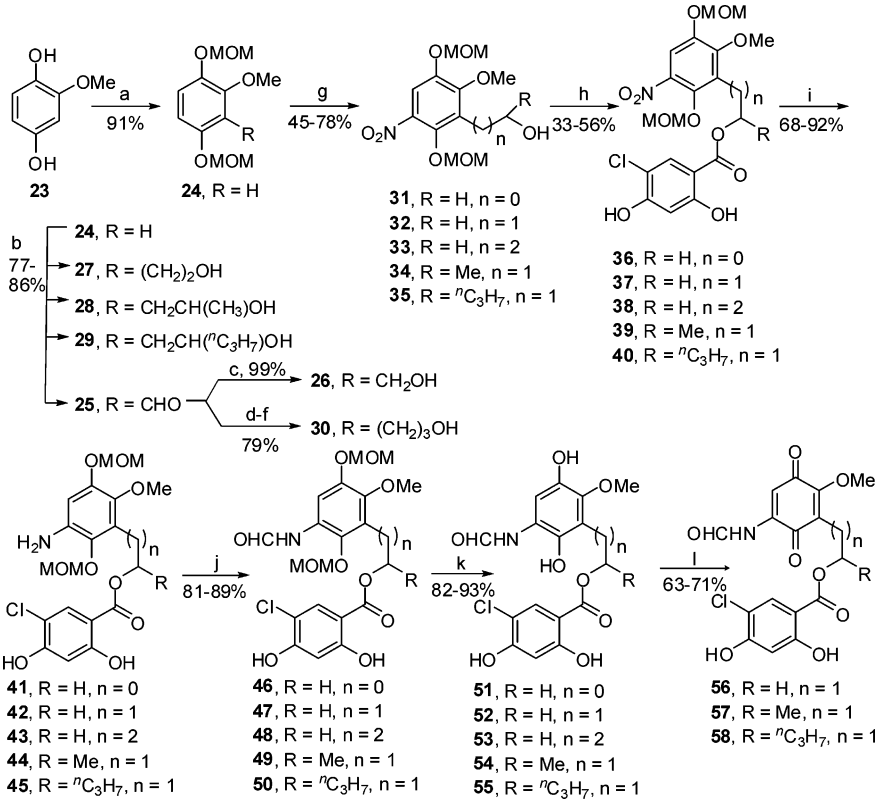
SCHEME 2. Synthesis of Radester Derivatives[a]



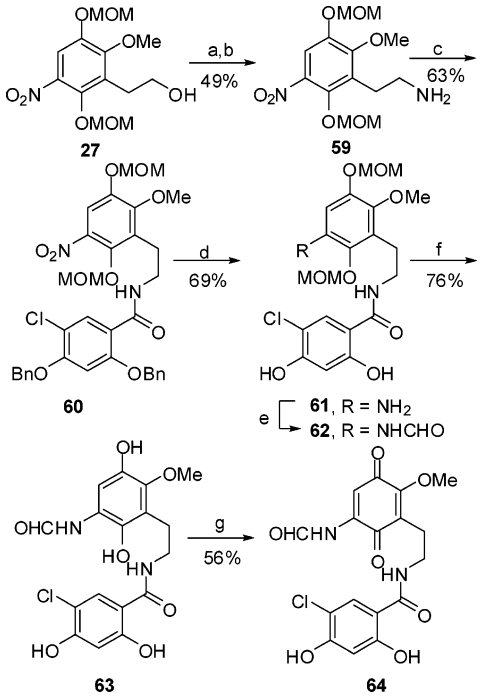
[a] Conditions: (a) NaH, MOMCl; (b) *n*-BuLi, E^+; (c) $NaBH_4$; (d) $(EtO)_2P(O)CH_2CO_2Et$; (e) H_2, Pd/C; (f) *i*-Bu_2AlH; (g) NH_4NO_3, $(CF_3CO)_2O$; LiOH, THF-MeOH-H_2O; (h) DCC, DMAP, 2,4-dihydroxy-5-chlorobenzoic acid, 50 °C; (i) H_2, PtO_2; (j) PhOCHO; (k) NaI, TMSCl; (l) O_2, Pd/C.

nitro group and formylation of the resulting anilines, gave **46**−**50**. Removal of the MOM protecting groups with in situ derived trimethylsilyl iodide furnished the corresponding hydroquinones, **51**−**55**.[15] Unlike previous attempts to oxidize the trimethoxy derivatives, oxidation of the hydroquinones with palladium on charcoal gave quinones **56**−**58** in good yield[39] (Scheme 2).

To furnish the amide derivatives, alcohol **27** was converted to the corresponding azide via a Mistunobu reaction with diphenyl phosphoryl azide.[40] Selective reduction of the azide in the presence of the nitro group was accomplished with sodium sulfide and triethylamine to afford compound **59**,[41] which was subsequently coupled with 2,4-bis(benzyloxy)-5-chlorobenzoic acid to provide amide **60**. Reduction of the nitro substituent gave aniline **61**. Formylation with phenylformate generated **62**. Removal of the MOM ethers with sodium iodide and trimethylsilyl chloride provided hydroquinone **63**, which was subsequently oxidized to the corresponding quinone **64** (Scheme 3).

To verify whether additional hydrophobic moieties could enhance binding to the π-rich pocket adjacent to the resorcinol moiety in which the chlorine atom of RDC projects, additional compounds were prepared for elucidation of potential binding interactions. For example, resorcinolic acid **1** was brominated or iodinated to generate the halogenated derivatives **65** and **66**.[42] After coupling with the secondary alcohol, reduction, formylation, and deprotection of the MOM groups, yielded hydro-

SCHEME 3. Synthesis of Radester Amide Analogs[a]



[a] Conditions: (a) DIAD, PPh_3, $(PhO)_2PON_3$; (b) Na_2S, Et_3N; (c) DCC, DMAP, 2,4-bis(benzyloxy)-5-chlorobenzoic acid, DMF-THF, 50 °C; (d) H_2, Pd/C; (e) PhOCHO; (f) NaI, TMSCl, CH_3CN-CH_2Cl_2; (g) O_2, Pd/C.

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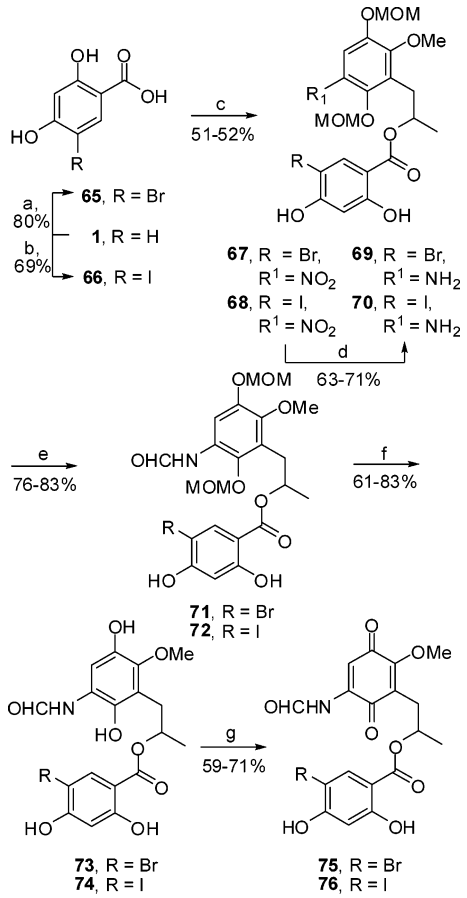
quinones **73** and **74**, which were oxidized to the corresponding quinones **75** and **76**. (Scheme 4).

SCHEME 4. Synthesis of 4-halogenated Derivatives[a]

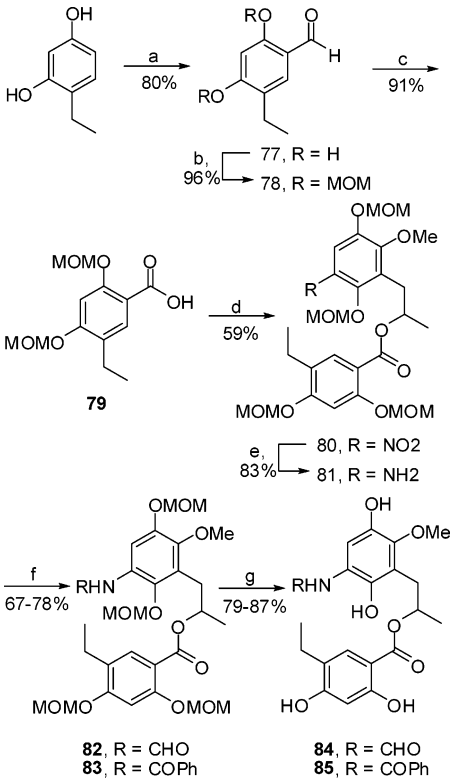


[a] Conditions: (a) Br_2, AcOH; (b) ICl; (c) DCC, DMAP, **34**, DMF-THF, 50 °C; (d) H_2, PtO_2 or $SnCl_2$, H_2O, dry EtOH; (e) PhOCHO; (f) NaI, TMSCl, CH_3CN-CH_2Cl_2; (g) O_2, Pd/C.

Likewise, 4-ethylresorcinol was converted into aldehyde **78** via a Vilsmeier−Haack reaction with phosphorus oxychloride and dimethylformamide,[43] which upon oxidation with sodium chlorite gave the acid, **79**.[44] After coupling, reduction, amidation, and removal of the MOM ethers, the hydroquinones **84** and **85** were produced (Scheme 5).

To determine the essential nature of each substituent on the quinone ring, several derivatives were prepared for elucidation of structure−activity relationships (SAR). 2-Methoxyphenol (**86**) and 3-methoxyphenol (**87**) were protected as the *t*-butyldiphenylsilyl and methoxy methyl ethers **88**[45] and **89**, respectively, which were then alkylated to generate **90** and **91**. The desired products **94** and **95** were furnished by coupling 2,4-dihydro-5-chlorobenzoic acid with alcohols **90** and **91**, followed by deprotection of *tert*-butyldiphenylsilyl and methoxymethyl ethers.[46] Aniline **44** was coupled with propanoic acid or benzoic acid to give amides **100** and **101**, respectively (Scheme 6).

SCHEME 5. Synthesis of Ethyl-substituted Radester[a]



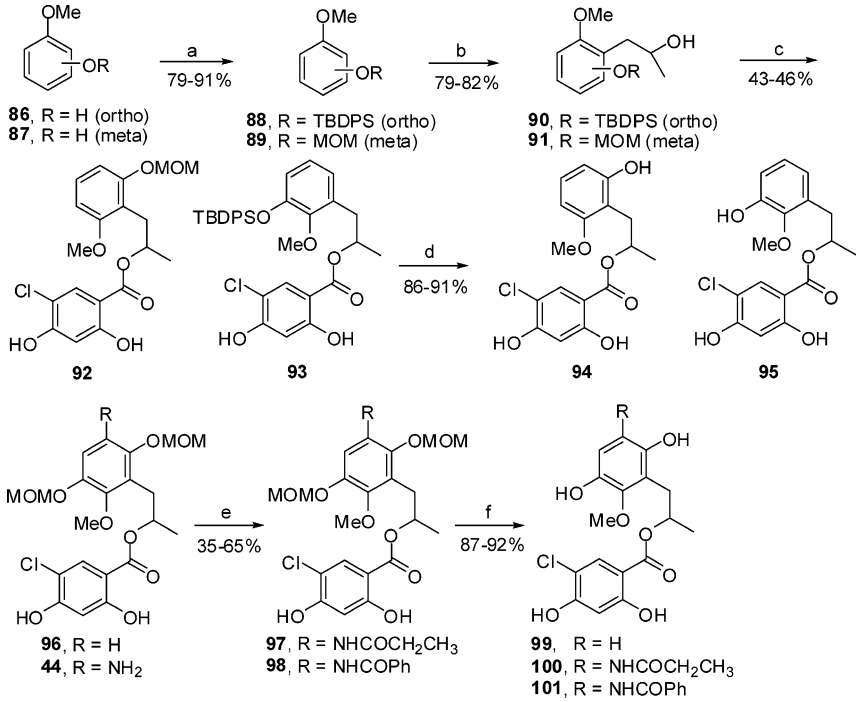
[a] Conditions: (a) $POCl_3$, DMF; (b) NaH, MOMCl; (c) $NaClO_2$, NaH_2PO_4; (d) DCC, DMAP, **34**, DMF-THF, 50 °C; (e) H_2, PtO_2; (f) PhOCHO or DCC, PhCOOH; (g) NaI, TMSCl, CH_3CN-CH_2Cl_2.

Radester Biological Studies. Upon completion of their syntheses, radester derivatives **11**−**22**, **51**−**58**, **63**, **64**, **73**−**76**, **84**, **85**, **94**, **95**, and **99**−**101** were evaluated for their antiproliferative activity against MCF7 breast cancer cells. The results indicated that the two-carbon trimethoxy aniline (**16**), isopropyl hydroquinone (**54**) and quinone (**57**), and isobutylquinone (**58**) were the most potent derivatives prepared and manifested IC_{50} values of 12.3, 7.1, 13.9, and 11.6 μM, respectively (Table 2). Such results indicate first that the hydroquinones are more potent than their quinone counterparts, which is best understood by the two hydroxyl groups acting as H-bond donors with Lys112 and Asp54. These results are consistent with Kosan1559[16] and NovartisG3130, as well as Infinity's compound,[25] which recently entered clinical trials as the hydroquinone. Second, the isopropyl linker is optimal for connecting the resorcinol and hydroquinone rings. Third, the chloro derivative is more active than the bromo, which is more active than the iodo, suggesting there is a limit to the size in which atoms can specifically bind in the π-rich pocket created by Phe124. Finally, the amide linker did not demonstrate increased potency compared with the ester linker, suggesting that potential H-bond acceptors are necessary in this region of the binding pocket.

Human epidermal growth factor receptor2 (Her2) is overexpressed in many breast cancers and is a well-known Hsp90-dependent substrate.[47] Therefore, the Her2 overexpressing cell line, SkBr3, was used for incubation with the chosen products (Table 2). Her2 levels were quantified following a protocol

(43) Zhang, Z.; Yu, B. *J. Org. Chem.* **2003**, *68*, 6309−6313.
(44) Makara, G. M.; Anderson, W. K. *J. Org. Chem.* **1995**, *60*, 5717−5718.
(45) Likubo, K. I.; Ando, N.; Umezawa, K.; Nishiyama, S. *Tetrahedron Lett.* **2002**, *43*, 291−293.
(46) Goujon, J. Y. Z.; Pagnocelli, S.; Boursereau, Y.; Kirschleger, B. *Synlett* **2002**, *2*, 322−324.
(47) Slamon, D. J.; Godolphin, W.; Jones, L. A.; Holt, J. A.; Wong, S. G.; Keith, D. E.; Levin, W. J.; Stuart, S. G.; Udove, J.; Ullrich, A. *Science* **1989**, *244*, 707−712.

SCHEME 6. Optimization of Radester Amide Side Chain[a]



[a] Conditions: (a) NaH, TBSCl; (b) *n*-BuLi, TMEDA, propylene oxide; (c) DCC, DMAP, 2,4-dihydroxy-5-chlorobenzoic acid, DMF-THF, 50 °C; (d) TBAF or NaI, TMSCl; (e) DCC, DMAP, PhCOOH, or CH_3CH_2COOH; (f) NaI, TMSCl.

developed by the Chiosis laboratory.[48] As shown, compounds **58**, **74**, **84**, **85**, **94**, **99**, **100**, and **101** proved most effective. The results indicate that the secondary ester derived from a two-carbon linker is superior when compared to the primary ester of varying length. In addition, the ester is more potent than the corresponding amide derivative. Furthermore, the 4-iodo compound is most potent and the simple formamide is more efficacious than larger, more hydrophobic moieties. Moreover, the concentration of inhibitors needed to induce the degradation of Her2 parallels the concentration needed to exhibit antiproliferative activity, clearly linking Hsp90 inhibition to cell viability for most compounds.

TABLE 2. Antiproliferative and Her2 Degradation Results[a]

entry	MCF7 (IC_{50}, μM)	Her2 (IC_{50}, μM)
11	31.4 ± 3.03	25.8 ± 2.97
12	38.7 ± 0.01	37.7 ± 5.01
13	17.2 ± 2.62	41.5 ± 12.2
14	27.2 ± 0.77	24.7 ± 0.81
15	>100	72.5 ± 10.6
16	12.3 ± 3.08	31.1 ± 4.43
17	41.4 ± 4.34	27.2 ± 1.27
18	>100	>80
19	45.0 ± 1.06	31.6 ± 0.03
20	69.1 ± 6.71	35.6 ± 2.04
21	46.8 ± 3.96	39.1 ± 2.92
22	77.7 ± 2.52	38.1 ± 6.01
51	18.0 ± 2.66	53.7 ± 17.0
52	51.6 ± 7.46	38.5 ± 1.86
53	40.4 ± 2.50	54.9 ± 2.45
54	7.06 ± 0.30	21.2 ± 6.17
55	18.2 ± 1.79	16.4 ± 5.09
56	26.3 ± 3.00	24.1 ± 1.52
57	13.9 ± 1.41	52.9 ± 6.54
58	11.6 ± 1.88	3.14 ± 0.79
63	>100	45.5 ± 0.53
64	17.0 ± 1.25	59.2 ± 10.5
73	38.6 ± 1.45	32.8 ± 8.38
74	24.7 ± 2.03	7.69 ± 1.21
75	33.1 ± 1.69	28.2 ± 2.22
76	>100	22.1 ± 2.61
84	5.72 ± 0.34	9.45 ± 4.55
85	8.95 ± 0.66	6.21 ± 1.97
94	69.4 ± 6.13	10.7 ± 2.07
95	>80	34.8 ± 8.99
99	5.71 ± 1.27	4.03 ± 0.46
100	>80	7.18 ± 4.01
101	4.27 ± 0.32	8.24 ± 0.07

[a] Antiproliferative assays were conducted in the MCF7 breast cancer cell line. Her2 degradation was performed in SkBr3 breast cancer cells. All values (μM) represent the average of $n = 3 \pm$ standard deviation.

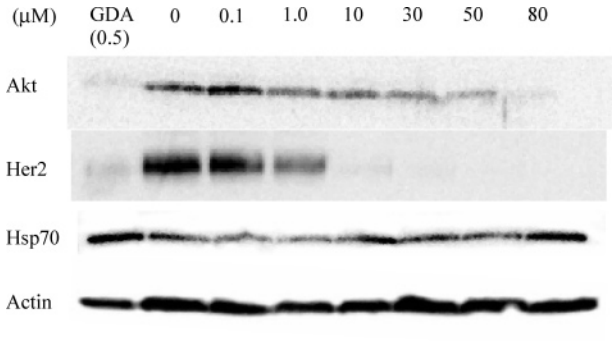


FIGURE 7. Western blot analysis of Hsp90 client protein degradation assay.

Because Hsp90 is responsible for the maturation of several client proteins, compound **101** was selected for incubation with the MCF7 breast cancer cell line for 24 h, upon which, the cells were harvested and the Hsp90 clients probed via western blot techniques (Figure 7). Analyses demonstrated that Akt levels

(48) Huezo, H.; Vilenchik, M.; Rosen, N.; Chiosis, G. *Chem. Biol.* **2003**, *10*, 629−634.

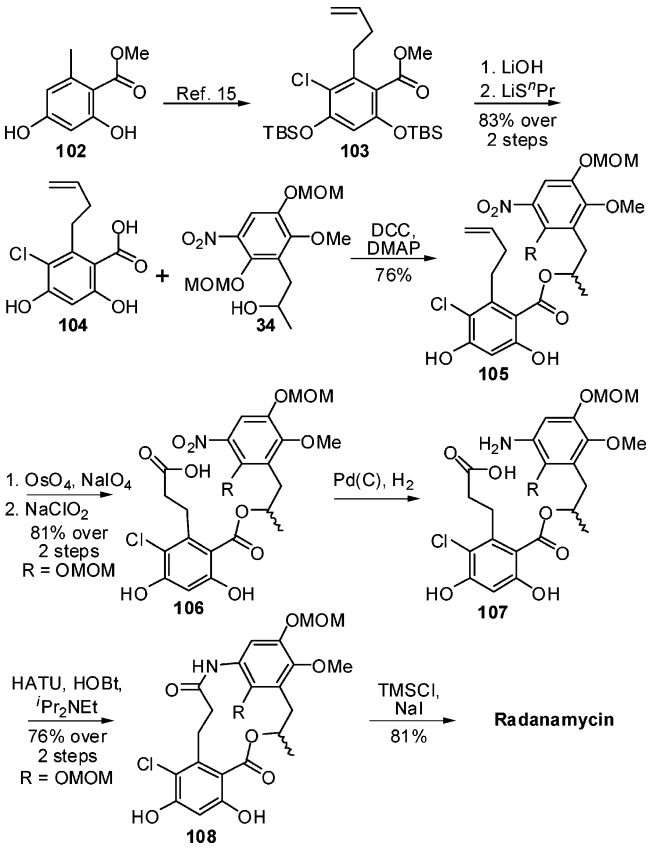
decreased as the concentration of inhibitor increased. Her2 was also degraded, producing IC_{50} values between 1.0 and 10 μM. In contrast, Hsp70 levels increased in a concentration-dependent manner, which is a hallmark of Hsp90 inhibition. Actin is not an Hsp90 client protein and remained unaffected by the concentration of inhibitor.

Radanamycin and Derivatives. As a starting point for the construction of a macrocyclic hybrid of radicicol and geldanamycin, we chose to prepare the compound that contained a three-carbon linker, as in the case of radamide,[24] along with the two-carbon linker found in radester[8] (primary and secondary alcohols) with and without the hydroquinone. Radester was shown to be a 7.06 μM inhibitor, whereas radamide remained approximately 6 times less active (42 μM).[8,24]

Radanamycin was prepared as previously disclosed and as shown in Scheme 7.[23] The resorcinolic methyl ester **102** was protected, chlorinated, and alkylated with allyl bromide to give **103** (Scheme 7). Removal of the protecting groups and hydrolysis of the ester afforded **104**, which was subsequently coupled with alcohol **34**[8] to furnish the corresponding ester, **105**. Oxidation of the alkene ultimately afforded acid **106**.[23] The nitro substituent was reduced and macrolactamization was attempted. Numerous procedures failed to give the macrocyclic product, however, the use of HOBt and HATU at elevated temperatures cleanly gave macrolactam **108** in good yield.[23] Removal of the MOM ethers gave the hydroquinone of radanamycin,[23] which was evaluated in MCF-7 breast cancer cells and found to exhibit an IC_{50} of 1.2 μM.

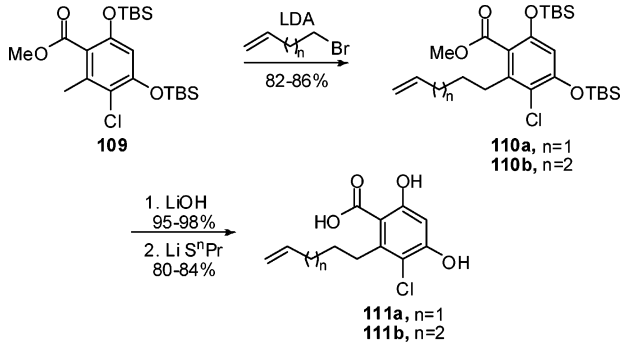
To determine whether a longer tether between the radamide quinone ring and the resorcinol moiety could provide more effective inhibitors, a systematic study was undertaken. Syntheses of the 4- and 5-carbon acids are given in Scheme 8.

SCHEME 7. Synthesis of Radanamycin



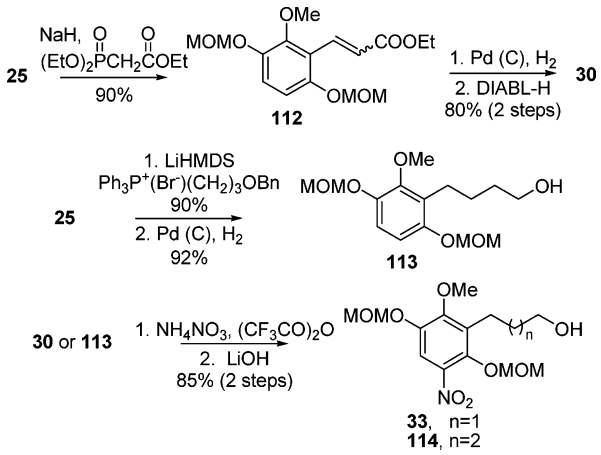
Instead of alkylation with allyl bromide, the protected resorcinol ring was extended to the desired carbon lengths by treatment with butenyl bromide or pentenyl bromide to provide **110a** or **110b**, respectively. Removal of the silyl ethers and hydrolysis of the esters gave acids **111a** and **111b** in good yields.

SCHEME 8. Syntheses of 4- and 5-Carbon Acid Analogues



Preliminary results confirmed that the 5-carbon analogue on the amide side chain proved most active for radamide, therefore, more flexible analogues were prepared and the number of methylenes on the ester side chain also increased. The alcohols used to prepare these compounds were also derived from the MOM-protected hydroquinone, **24** (Scheme 9). However, instead of alkylation with propylene oxide, the requisite lithium anion was treated with *N*,*N*-dimethylformamide to furnish the corresponding aldehyde, **25**. The aldehyde was then treated with the sodium anion of triethylphosphonoacetate to give the α,β-unsaturated ester **112**. Similarly, **25** was treated with the ylide of triphenylphosphonium 4-benzyloxybutane bromide to afford the benzyl-protected olefinic compound. Reduction of the α,β-unsaturated ester proceeded in two steps to afford the 3-carbon alcohol **30**. Likewise the 4-carbon derivative was prepared in one step by simultaneous reduction of both the benzyl protecting group and the alkene. Individually, the alcoholic aromatics were nitrated[49] to furnish the necessary substrates (**33** and **114**) for subsequent coupling with the 5-carbon olefinic acid, **111b**.

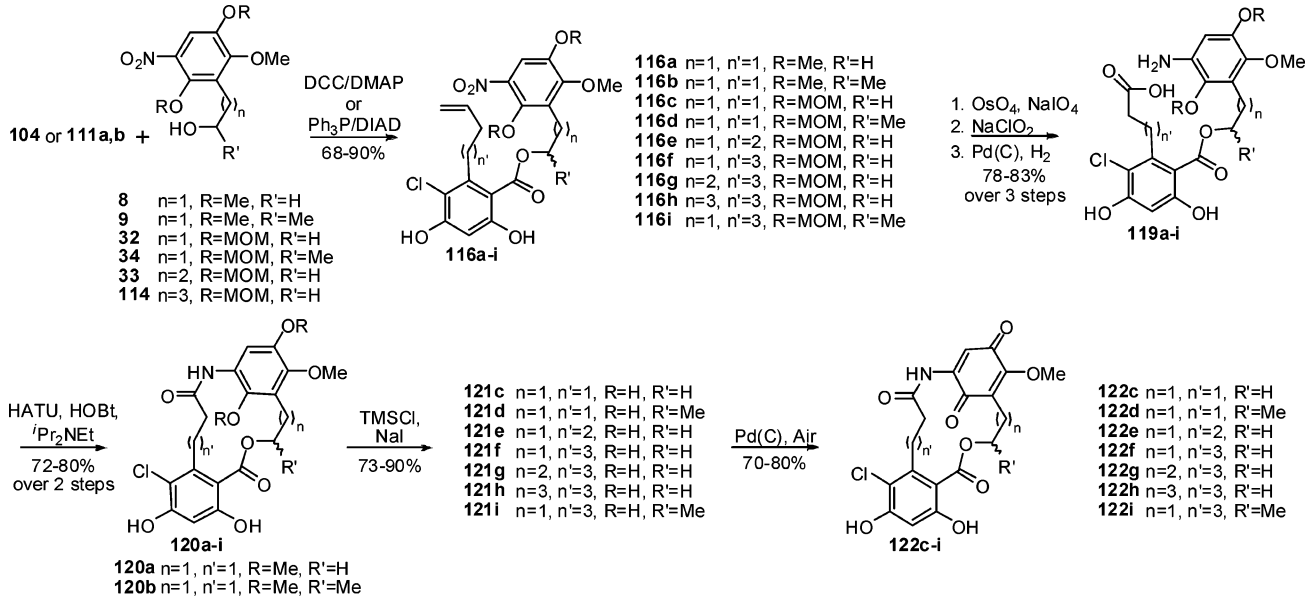
SCHEME 9. Syntheses of 3- and 4-Carbon Alcohol Analogues



As shown in Scheme 10, alcohols **8**, **9**, **32**−**34**, and **114** were coupled with the corresponding acids (**104** or **111a** and **111b**) via Mitsunobu esterification or DCC-mediated coupling to pro-

(49) Crivello, J. V. *J. Org. Chem.* **1981**, *46*, 3056.

SCHEME 10. Synthesis of 2,4,5-Trimethoxy and Hydroquinone Species



vide the desired products **116a**−**i**. The olefins were oxidized to the corresponding acids, and the nitro substituents were reduced to afford the aniline products **119a**−**i** before macrocyclization was attempted. Substantial effort was invested in this step, but numerous conditions failed to give the desired macrolactams. Eventually, conditions were identified that provided the macrocycles **120a**−**i** in good yield through the implementation of HATU and HOBt. The hydroquinone was unmasked by removal of the MOM-protecting groups to afford **121c**−**i**, then oxidized to the corresponding quinones, **122c**−**i**.[15,50]

Upon completion of their syntheses, both the trimethoxy (**120a** and **120b**) and the hydroquinone (**121c**−**i**) species were evaluated for antiproliferative activity against MCF-7 breast cancer cells (Table 3). Consistent with earlier studies reported by Andrus and co-workers,[15] the trimethoxy compound (**120b**) was shown to be substantially less active than the hydroquinone, as evidenced by Table 3. The data clearly show that, similar to radester, the macrocycle **121d** derived from the secondary alcohol is more potent than its desmethyl counterpart, **121c**.

TABLE 3. Macrocyclic IC_{50} Values[a]

entry	MCF7 (IC_{50}, μM)	Her2 (IC_{50}, μM)
120b	41.0 ± 3.69	2.98 ± 0.33
121c	2.85 ± 0.78	5.32 ± 0.13
121d	1.21 ± 0.08	2.54 ± 0.45
121e	3.46 ± 1.16	3.77 ± 0.33
121f	2.47 ± 0.18	3.77 ± 0.79
121g	0.94 ± 0.07	4.54 ± 1.37
121h	1.62 ± 0.21	1.32 ± 0.03
121i	0.93 ± 0.17	1.25 ± 0.03
122c	15.4 ± 0.8	20.6 ± 0.08
122d	9.65 ± 0.55	14.4 ± 5.27
122e	5.95 ± 0.31	4.25 ± 0.11
122f	1.61 ± 0.35	4.71 ± 0.46
122g	2.63 ± 0.53	3.91 ± 0.57
122h	2.85 ± 0.74	4.03 ± 0.21
122i	1.10 ± 0.05	1.91 ± 0.27

[a] Antiproliferative assays were conducted in the MCF-7 breast cancer cell line. Her2 degradation was performed in SkBr3 breast cancer cells. All values (μM) represent the average of $n = 3 \pm$ standard deviation.

(50) Luly, J. R.; Rapoport, H. *J. Org. Chem.* **1984**, *49*, 1671.

Radanamycin Biological Studies. To verify that the antiproliferative activity for these compounds was related to Hsp90 inhibition, the macrocycles were incubated with the Her2 overexpressing breast cancer cell line, SKBr3. Following the procedure of Chiosis and co-workers,[48] the Her2 levels were quantified after 24 h. As can be seen in Table 3, the IC_{50} values obtained from this assay mirrored the corresponding values from the antiproliferative studies, clearly linking abolishment of the Hsp90-dependent protein Her2, to cell viability.

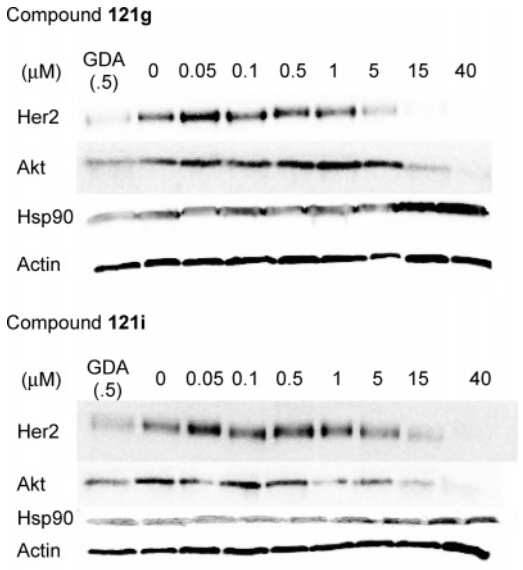


FIGURE 8. Western blot analyses of **121g** and **121i** in SKBr3 breast cancer cells.

Subsequent Western blot analysis of **121g** and **121i** in SKBr3 breast cancer cells at increasing concentrations for 24 h was performed. As can be seen in Figure 8, **121g** and **121i** had significant effects at 1 μM, as Hsp90-dependent client proteins[1] (Her2, Raf1, and Akt) were readily degraded in the presence of these compounds. Similar to other Hsp90 inhibitors, both **121g** and **121i** caused induction of Hsp90 in a concentration-

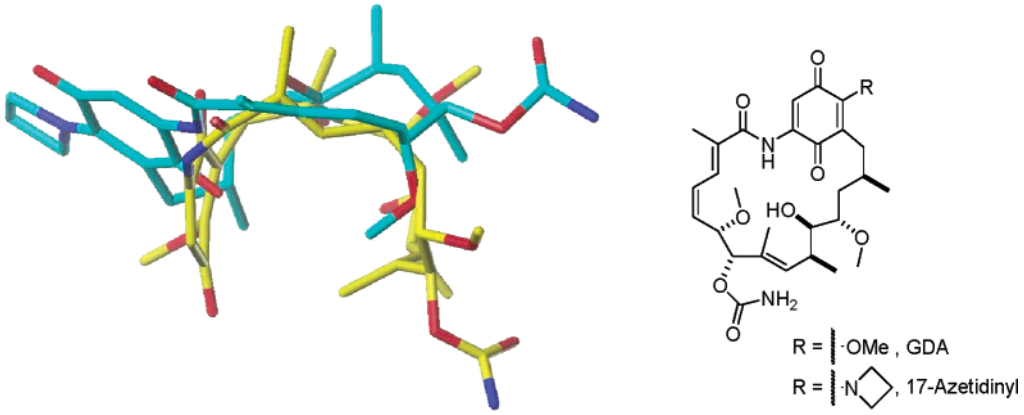


FIGURE 9. Comparison of native GDA crystal structure (cyan) and bound conformation of GDA to Hsp90 (yellow).

dependent manner.[51] Actin is not an Hsp90-dependent substrate and remained unaffected by Hsp90 inhibition, thereby allowing a suitable control for comparison of relative protein levels.

Intrigued by the above findings, we re-evaluated the previously solved cocrystal structures of RDC and GDA bound to Hsp90 (Figure 3) and realized there were two possible orientations for the macrocyclic products, one that overlapped with the epoxide of RDC[5] and another that extended from the interior of the pocket and occupied the region that bound the quinone ring of GDA.[19] Therefore, we sought to identify the optimal chain length connecting the resorcinol ring of RDC to the quinone of GDA in an effort to increase the potency of radamide and provide a starting point for the construction of additional macrocyclic compounds. Results from these studies clearly showed that a tether containing a five-carbon linker between the resorcinol ring and the quinone increased activity and provided a radamide analogue with approximately the same potency as the previously identified inhibitor, radester.

In analogous fashion, the optimal linker length for radester was determined to identify the two-carbon linker as the most-active compound. However, potency was further increased by introduction of a secondary alcoholic ester in lieu of the primary alcohol. Not surprisingly, a similar substitution is found in both natural product inhibitors of Hsp90. With these results in hand, the pursuit of larger macrocyclic chimeras was commenced to vary the ring size from 13 to 17 carbons by incorporation of additional methylene groups into the linker side chains of the macrocycle.

Consistent with our earlier results, macrocyclic compounds **121g** and **121i** showed increased activity against MCF-7 breast cancer cells, suggesting that compounds with a more flexible linker provide greater inhibitory activity. Cocrystal structures of geldanamycin bound to Hsp90 have shown that the amide bond is isomerized to the *cis*-isomer,[19] which is distinctive from its native crystal structure that clearly shows the *trans*-amide species[52] (Figure 9). Both Santi[53] and Neckers[54] have put forth potential explanations to rationalize the observed mode of binding by computational and site-directed mutagenesis studies, respectively. The Santi studies propose that the quinone moiety must rotate in the Hsp90 ATP binding site to accommodate its final resting place before isomerization. We believe that, in the case of compounds with two methylene units in each tether, the macrocycles are too rigid to first rotate and, thus, the hydroquinone moiety binds in a similar spatial orientation as the radicicol epoxide. However, by increasing the tether length between the resorcinol ring and the hydroquinone, the molecule becomes flexible enough to undergo this rotation and eventually occupies the appropriate binding pocket. However, further tether elongation to afford compound **120b** resulted in significantly reduced activity, suggesting there is a limit to the ring size recognized by Hsp90. Verification of this mode of binding is currently under investigation through crystallographic studies.

Results from these studies indicate that chimeric compounds composed of RDC's resorcinol ring and GDA's quinone ring produce potent Hsp90 inhibitors, and further derivatization of these molecules is likely to afford analogues with increased activity and, perhaps, useful alternatives to the geldanamycin derivatives that are currently in 26 clinical trials. Additional studies with these chimeric compounds are in progress and additional structure−activity relationships for these agents will be reported in due course.

Conclusion

Several series of chimeric compounds were prepared based on the cocrystal structures of natural product inhibitors of Hsp90, geldanamycin, and radicicol. Molecular modeling experiments supported modification of radester and radanamycin to increase Hsp90 inhibitory activity. Upon preparation of these compounds, antiproliferative assays and protein degradation assays supported the initial hypothesis that chimeric compounds represent good lead compounds for the development of Hsp90 inhibitors. Through the preparation of several analogues, the first structure−activity relationships have been produced for radanamycin and its seco-agent, radester, and have laid the foundation for more efficacious compounds.

Experimental Section

1-(2-Methoxy-3,6-bis(methoxymethoxy)phenyl)pentan-2-ol (29). 2-Methoxy-1,4-bis-methoxymethoxy-benzene (1 g, 4.4 mmol) was dissolved in THF (44 mL) before addition of TMEDA (0.8 mL, 5.3 mmol). The solution was cooled to 0 °C, and *n*-BuLi (3.3 mL, 5.3 mmol) was added dropwise. The mixture was stirred at room temperature for 4 h before it was cooled to 0 °C, and 1,2-

(51) Chiosis, G.; Vilenchik, M.; Kim, J.; Solit, D. *Drug Discovery Today* **2004**, *9*, 881.
(52) Schnur, R. C.; Corman, M. L. *J. Org. Chem.* **1994**, *59*, 2581−2584.
(53) Jez, J. M.; Chen, J. C.-H.; Rastelli, G.; Stroud, R. M.; Santi, D. V. *Chem. Biol.* **2003**, *10*, 361.
(54) Lee, Y.-S.; Marcu, M. G.; Neckers, L. M. *Chem. Biol.* **2004**, *11*, 991.

epoxypentane (0.91 mL, 8.8 mmol) was added. After stirring for 2 h, the reaction was quenched with 0.5 M HCl solution until the pH = 7. The mixture was extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 15% EtOAc in hexanes) to give **29** as a yellow oil (1.18 g, 86%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.96 (d, J = 9.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 1H), 5.14 (s, 2H), 5.12 (s, 2H), 3.86 (s, 3H), 3.81 (m, 1H), 3.51 (s, 3H), 3.47 (s, 3H), 2.94 (m, 1H), 2.78 (m, 1H), 2.56 (s, OH), 1.47 (m, 4H), 0.94 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.3, 149.4, 145.7, 123.3, 115.7, 110.0, 96.1, 95.4, 72.6, 61.1, 56.5, 56.4, 40.2, 32.5, 19.4, 14.5; IR (film) ν_{max} 3583, 3053, 2958, 1593, 1465, 1205, 1153, 1056, 946 cm^{-1}; HRMS (ES$^+$) m/z: [M + H] calcd for $C_{16}H_{27}O_6$, 315.1808; found, 315.1811.

1-(2-Methoxy-3,6-bis(methoxymethoxy)-5-nitrophenyl)pentan-2-ol (35). Compound **29** (0.84 g, 2.7 mmol) was dissolved in CH_3CN (10 mL) and stirred at −10 °C before addition of ammonium nitrate (749 mg, 9.2 mmol). $(CF_3CO)_2O$ (11.7 mL, 9.2 mmol) was added to the mixture, and it stirred for 15 min at room temperature. The mixture was quenched by addition of saturated aqueous sodium bicarbonate (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved with THF−MeOH−H_2O (3:1:1, 15 mL) before LiOH (276 mg, 11.5 mmol) was added and stirred for 5 min. The mixture was quenched by addition of saturated aqueous sodium bicarbonate (5 mL) and extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 20% EtOAc in hexanes) to give **35** as a brown oil (557 mg, 58%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.66 (s, 1H), 5.24 (s, 2H), 5.12 (d, J = 6.4 Hz, 1H), 5.05 (d, J = 6.4 Hz, 1H), 3.97 (s, 3H), 3.87 (m, 1H), 3.61 (s, 3H), 3.53 (s, 3H), 2.91 (m, 2H), 2.46 (m, OH), 1.55 (m, 2H), 1.45 (m, 2H), 0.97 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.4, 146.6, 146.4, 139.8, 130.1, 111.7, 102.3, 95.8, 71.8, 61.4, 58.0, 56.9, 41.0, 33.4, 19.3, 14.5; IR (film) ν_{max} 3468, 3053, 2985, 1578, 1477, 1155, 1022, 897 cm^{-1}; HRMS (FAB$^+$) m/z: [M + H] calcd for $C_{16}H_{26}NO_8$, 360.1658; found, 360.1672.

1-(2-Methoxy-3,6-bis(methoxymethoxy)-5-nitrophenyl)pentan-2-yl 5-chloro-2,4-dihydroxybenzoate (40). 2,4-Dihydroxy-5-chloro-benzoic acid (209 mg, 1.1 mmol) was dissolved in DMF/THF (1:3, 11 mL) before DMAP (201 mg, 1.65 mmol), DCC (340 mg, 1.65 mmol), and compound **35** (400 mg, 1.1 mmol) were added. The reaction was warmed to 50 °C and stirred for 12 h. The mixture was quenched by addition of saturated aqueous ammonium chloride (20 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 10% EtOAc in hexanes) to give **40** as a pale yellow solid (301 mg, 51%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (s, 1H), 7.63 (s, 1H), 6.57 (s, 1H), 5.42 (m, 1H), 5.19 (m, 2H), 5.06 (m, 2H), 4.00 (s, 3H), 3.61 (s, 3H), 3.50 (s, 3H), 3.21 (m, 1H), 3.11 (m, 1H), 1.69 (m, 2H), 1.45 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1, 162.8, 157.5, 153.6, 146.2, 139.6, 130.7, 130.6, 127.9, 112.4, 111.6, 107.2, 104.3, 102.2, 95.9, 75.2, 61.6, 58.3, 56.9, 36.7, 30.1, 19.1, 14.3; IR (film) ν_{max} 3055, 2986, 1668, 1525, 1421, 1265, 1157 cm^{-1}; HRMS (ES$^+$) m/z: [M + H] calcd for $C_{23}H_{29}NO_{11}Cl$, 530.1429; found, 530.1426.

1-(3-Amino-6-methoxy-2,5-bis(methoxymethoxy)phenyl)pentan-2-yl 5-chloro-2,4-dihydroxybenzoate (45). Compound **40** (200 mg, 0.38 mmol) was dissolved in ethanol (3.8 mL) at the room temperature before PtO_2 (10 mg, 5% w/w) was added. The reaction was stirred under a hydrogen atmosphere for 12 h. The solution was filtered through Celite and the filtrate was concentrated. The residue was purified via column chromatography (SiO_2, 10% EtOAc in hexanes) to give **45** as a brown solid (158 mg, 84%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (s, 1H), 6.57 (s, 1H), 6.50 (s, 1H), 5.42 (m, 1H), 5.14 (m, 2H), 4.99 (m, 2H), 3.81 (s, 3H), 3.63 (s, 3H), 3.49 (s, 3H), 3.03 (m, 2H), 1.65 (m, 2H), 1.39 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 162.6, 157.6, 147.8, 141.0, 139.3, 136.3, 130.9, 125.6, 111.6, 107.4, 104.3, 104.1, 100.1, 95.8, 75.8, 61.4, 57.9, 56.6, 36.4, 30.1, 19.2, 14.3; IR (film) ν_{max} 3518, 3053, 2939, 1697, 1517, 1155, 1036, 894 cm^{-1}; HRMS (ES$^+$) m/z: [M + H] calcd for $C_{23}H_{31}ClNO_9$, 500.1687; found, 500.1691.

1-(3-Formamido-6-methoxy-2,5-bis(methoxymethoxy)phenyl)-pentan-2-yl 5-chloro-2,4-dihydroxybenzoate (50). Compound **45** (200 mg, 0.40 mmol) was dissolved in CH_2Cl_2 (40 mL) at 0 °C before PhOCHO (182 mg, 1.5 mmol) was added. The reaction was warmed to 30 °C and stirred for 12 h. After evaporation of organic solvent, the residue was purified via column chromatography (SiO_2, 10% EtOAc in hexanes) to give **50** as a yellow solid (177 mg, 84%) as a mixture of rotamers: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 1H), 8.09 (s, 1H), 7.84 (s, 1H), 6.58 (s, 1H), 5.39 (m, 1H), 5.18 (m, 2H), 5.00 (m, 2H), 3.89 (s, 3H), 3.62 (s, 3H), 3.51 (s, 3H), 3.03 (d, J = 6.7 Hz, 2H), 1.64 (m, 2H), 1.38 (m, 2H), 0.94 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.2, 169.1, 162.7, 162.6, 159.3, 157.7, 157.6, 147.8, 147.0, 146.7, 145.6, 143.2, 141.8, 130.7, 126.9, 126.6, 125.0, 111.6, 109.6, 107.3, 107.2, 107.1, 104.4, 101.2, 101.1, 96.0, 95.9, 75.5, 75.3, 61.4, 58.0, 57.8, 56.9, 56.8, 36.5, 36.3, 30.2, 30.1, 19.2, 14.3; IR (film) ν_{max} 3392, 2986, 1697, 1529, 1396, 1155, 1113, 960 cm^{-1}; HRMS (ES$^+$) m/z: [M + H] calcd for $C_{24}H_{31}NO_{10}Cl$, 528.1636; found, 528.1644.

1-(3-Formamido-2,5-dihydroxy-6-methoxyphenyl)pentan-2-yl 5-chloro-2,4-dihydroxybenzoate (55). Ester **50** (30 mg, 0.057 mmol) was dissolved in CH_2Cl_2 (0.3 mL) and CH_3CN (0.3 mL) at 25 °C before addition of sodium iodide (85 mg, 0.57 mmol) and trimethylsilyl chloride (72.4 μM, 0.57 mmol). The turbid solution was stirred for 15 min before saturated $Na_2S_2O_3$ (10 mL) was added. The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 50% EtOAc in hexanes) to afford **55** (21.7 mg, 87%) as a red oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.72 (s, 1H), 8.30 (s, 1H), 7.86 (m, 3H), 7.31 (s, 1H), 6.62 (s, 1H), 6.40 (s, 1H), 5.96 (s, 1H), 5.10 (m, 1H), 3.82 (s, 3H), 3.16 (m, 1H), 3.05 (m, 1H), 1.74 (m, 2H), 1.51 (m, 1H), 1.36 (m, 1H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 162.8, 160.1, 157.9, 144.0, 142.8, 139.9, 130.7, 121.9, 119.4, 112.0, 108.2, 106.9, 104.6, 76.8, 61.7, 36.0, 30.0, 19.3, 14.3; IR (film) ν_{max} 3512, 3392, 3055, 2985, 1618, 1373, 1161, 1045, 896 cm^{-1}; HRMS (ES$^+$) m/z: [M + H] calcd for $C_{20}H_{23}NO_8Cl$, 440.1112; found, 440.1134.

1-(5-Formamido-2-methoxy-3,6-dioxocyclohexa-1,4-dienyl)-pentan-2-yl 5-chloro-2,4-dihydroxybenzoate (58). Compound **55** (17 mg, 0.039 mmol) was dissolved in EtOAc (0.4 mL) before $Pd(OAc)_2$ (34 mg, 200% w/w) was added and stirred for 30 min. The mixture was filtered through silica gel pad, the filtrate was concentrated, and the residue was purified via prepared TLC (SiO_2, 250 μM, 50% EtOAc in hexane) to afford **58** (11.5 mg, 68%) as a brown solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.80 (s, 1H), 8.59 (a, 1H), 8.23 (s, 1H), 7.74 (s, 1H), 7.36 (s, 1H), 6.60 (s, 1H), 5.26 (m, 1H), 4.14 (s, 3H), 3.78 (m, 1H), 2.82 (m, 1H), 2.64 (m, 1H), 1.66 (m, 2H), 1.42 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183.7, 182.5, 168.7, 162.5, 159.4, 157.1, 136.8, 130.0, 129.9, 122.9, 114.2, 111.2, 106.7, 104.2, 74.2, 61.8, 36.5, 27.9, 18.7, 14.2; IR (film) ν_{max} 3603, 3398, 2972, 1664, 1616, 1379, 1161, 1122, 946 cm^{-1}; HRMS (ES$^+$) m/z: [M + NH_4] calcd for $C_{20}H_{24}N_2O_8Cl$, 455.1221; found, 455.1218.

3-(2-Azidoethyl)-2-methoxy-1,4-bis(methoxymethoxy)-5-nitrobenzene (59.1). Compound **27** (1.2 g, 4.41 mmol), triphenylphosphine (2.87 g, 11.0 mmol), and diisopropylazodicarboxylate (2.16 mL, 11.0 mmol) were dissolved in THF (20 mL) and stirred at 0 °C for 15 min before diphenyl phosphoryl azide (2.4 mL, 11.0 mmol) was added and stirred at room temperature for 12 h. The reaction was quenched by addition of saturated aqueous ammonium chloride (100 mL). The organic layer was removed, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined

organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 15% EtOAc in hexanes), affording **59.1** (1.46 g, 97%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (s, 1H), 5.24 (s, 2H), 5.07 (s, 2H), 4.00 (s, 3H), 3.60 (s, 3H), 3.53 (s, 3H), 3.47 (t, J = 7.6 Hz, 2H), 3.06 (t, J = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.6, 146.5, 146.3, 139.6, 128.4, 112.2, 102.5, 95.8, 61.7, 58.2, 57.0, 50.7, 25.3; IR (film) ν_{max} 3053, 2986, 2253, 2102, 1526, 1477, 1357 cm^{-1}; HRMS (ES$^+$) *m*/*z*: [M + Na] calcd for $C_{13}H_{18}N_4O_7Na$, 365.1073; found, 365.1068.

2-(2-Methoxy-3,6-bis(methoxymethoxy)-5-nitrophenyl)ethanamine (59). The azide **59.1** (0.33 g, 0.96 mmol) and sodium sulfide (0.487 g, 2.02 mmol) were dissolved in methanol (3.3 mL) before triethylamine (14 μL, 0.096 mmol) was added, and the mixture was stirred for 12 h before the addition of saturated aqueous ammonium chloride (30 mL). The organic layer was removed, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 30% EtOAc in hexanes), affording **59** (0.15 g, 51%) as the yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.54 (s, 1H), 5.17 (s, 2H), 4.98 (s, 2H), 3.81 (s, 3H), 3.64 (s, 3H), 3.54 (s, 3H), 3.46 (t, J = 7.7 Hz, 2H), 2.96 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.9, 140.6, 138.9, 136.6, 126.0, 103.7, 100.1, 95.8, 61.6, 57.9, 56.6, 51.3, 25.3; IR (film) ν_{max} 3448, 3373, 2935, 2902, 1612, 1491, 1155, 1059 cm^{-1}; HRMS (ES$^+$) *m*/*z*: [M + H] calcd for $C_{13}H_{21}N_2O_7$, 317.1349; found, 317.1352.

5-Bromo-2,4-dihydroxybenzoic Acid (65). 2,4-Dihydroxybenzoic acid (3 g, 19.6 mmol) was dissolved in acetic acid (21 mL) before bromine (1.1 mL, 23.3 mmol) dissolved in acetic acid (18 mL) was added dropwise and stirred for 4 h. The mixture was diluted with water (100 mL) and quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted rigorously with EtOAc until the extracts showed no sign of product. The combined EtOAc layers were dried over Na_2SO_4, filtered, and concentrated. The solid was recrystallized from a 50% acetonitrile in toluene to afford **65** as a yellow solid (3.6 g, 80%): ^{1}H NMR (DMSO, 400 MHz) δ 7.79 (s, 1H), 6.44 (s, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 171.3, 163.0, 159.7, 134.3, 108.7, 103.7, 99.2; IR (film) ν_{max} 3495, 3350, 1654, 1612, 1186, 1159, 1045, 842 cm^{-1}; HRMS (ES$^-$) *m*/*z*: [M − H] calcd for $C_7H_4BrO_4$, 230.9293; found, 230.9291.

5-Ethyl-2,4-bis(methoxymethoxy)benzaldehyde (78). Dimethylformamide (3 mL, 38.2 mmol) and phosphorus oxychloride (4 mL, 43.1 mmol) were mixed at 0 °C and stirred for 15 min before 2,4-dihydroxy-5-ethylbenzene (2 g, 14.5 mmol) dissolved in dimethylformamide (5 mL) was added. The mixture was stirred for 10 h and quenched by the addition of saturated aqueous sodium bicarbonate (100 mL). The solution was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in THF (120 mL), and the solution was cooled to 0 °C before NaH (60% in mineral oil, 2.32 g, 58 mmol) was cautiously added. The slurry was stirred for 2 h at room temperature before the addition of MOMCl (967 μL, 58 mmol). The mixture was stirred at room temperature for 4 h before quenching by the addition of saturated aqueous ammonium chloride (100 mL). The solution was extracted with EtOAc (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 10% EtOAc in hexanes) to give **78** as a brown oil (2.83 mg, 77% for two steps): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.30 (s, 1H), 7.66 (s, 1H), 6.88 (s, 1H), 5.26 (s, 4H), 3.52 (s, 3H), 3.49 (s, 3H), 2.59 (q, J = 7.5 Hz, 2H), 1.18 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.0, 161.6, 160.4, 128.9, 127.6, 120.0, 100.7, 95.3, 94.5, 57.0, 56.8, 23.1, 14.5; IR (film) ν_{max} 3055, 2935, 2873, 2773, 1606, 1581, 1396, 1209, 1116, 1057, 993 cm^{-1}; HRMS (ES$^+$) *m*/*z*: [M + NH_4] calcd for $C_{13}H_{22}NO_5$, 272.1498; found, 272.1495.

1-(3-Benzamido-6-methoxy-2,5-bis(methoxymethoxy)phenyl)propan-2-yl 5-chloro-2,4-dihydroxybenzoate (98). Compound **44** (80 mg, 0.17 mmol) was dissolved in THF (1.7 mL) before DCC (52.5 mg, 0.25 mmol) and benzoic acid (27 mg, 0.22 mmol) were added. The reaction was warmed to 50 °C and stirred for 12 h before the addition of saturated aqueous ammonium chloride (5 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 10% EtOAc in hexanes) to give **98** as a pale yellow solid (63 mg, 65%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.90 (s, 1H), 8.21 (d, J = 7.1 Hz, 1H), 8.06 (s, 1H), 7.66 (d, J = 7.4 Hz, 1H), 7.53 (m, 2H), 6.92 (s, 1H), 6.50 (s, 1H), 5.44 (m, 1H), 5.11 (m, 2H), 4.98 (m, 2H), 3.80 (s, 3H), 3.62 (s, 3H), 3.49 (s, 3H), 3.10 (m, 1H), 3.00 (m, 1H), 1.39 (d, J = 6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.8, 164.0, 161.5, 152.6, 147.8, 140.8, 139.1, 136.6, 134.5, 131.8, 130.9, 129.1, 128.9, 125.3, 117.6, 113.2, 112.5, 104.0, 100.0, 95.8, 73.6, 61.4, 57.9, 56.6, 31.5, 20.2; IR (film) ν_{max} 3327, 3053, 2935, 1697, 1610, 1375, 1110, 1049, 972 cm^{-1}; HRMS (ES$^+$) *m*/*z*: [M + H] calcd for $C_{28}H_{31}NO_{10}Cl$, 576.1636; found, 576.1629.

1-(3-Benzamido-2,5-dihydroxy-6-methoxyphenyl)propan-2-yl 5-chloro-2,4-dihydroxybenzoate (101). Ester **98** (20 mg, 0.035 mmol) was dissolved in CH_2Cl_2 (0.2 mL) and CH_3CN (0.2 mL) at 25 °C before the addition of sodium iodide (52 mg, 0.35 mmol) and trimethylsilyl chloride (45 μM, 0.35 mmol). The turbid solution was stirred for 15 min before saturated $Na_2S_2O_3$ (10 mL) was added to the mixture. The aqueous layer was extracted with EtOAc (3 × 30 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified via column chromatography (SiO_2, 50% EtOAc in hexanes) to afford **101** (15.4 mg, 91%) as a brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.90 (s, 1H), 8.21 (m, 2H), 7.89 (s, 1H), 7.67 (m, 1H), 7.54 (m, 2H), 6.94 (s, 1H), 5.53 (s, 1H), 5.31 (m, 1H), 5.08 (s, OH), 4.19 (s, 3H), 2.91 (m, 1H), 2.76 (m, 1H), 1.42 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.8, 182.3, 168.8, 164.0, 161.6, 159.0, 152.8, 147.1, 134.5, 131.5, 130.9, 129.1, 128.8, 121.6, 117.8, 113.4, 112.1, 100.5, 72.4, 62.3, 29.5, 20.5; IR (film) ν_{max} 3510, 3390, 2933, 1745, 1676, 1593, 1479, 1211, 908 cm^{-1}; HRMS (ES$^+$) *m*/*z*: [M − H] calcd for $C_{24}H_{21}NO_8Cl$, 486.0956; found, 486.0955.

2,3,6-Trimethoxy-5-nitrophenethyl-2-(but-3-enyl)-3-chloro-4,6-dihydroxybenzoate (116a). To a mixture of **8** (155 mg, 0.6 mmol) and PPh_3 (157 mg, 0.6 mmol) in THF (4 mL) was added diisopropyl azodicarboxylate (121 mg, 0.6 mmol) at 0 °C. After 10 min, a solution of **104** (150 mg, 0.6 mmol) in THF (1 mL) was added to the mixture at 0 °C and warmed to room temperature, stirring for 10 h. The solvent was removed under reduced pressure, and the residue was purified via chromatography (SiO_2, 20% EtOAc in hexanes) to afford **116a** (250 mg, 87%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (s, 1H), 6.54 (s, 1H), 5.78 (m, 1H), 4.97 (m, 2H), 4.50 (t, J = 7.4 Hz, 2H), 3.97 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.18 (t, J = 7.4 Hz, 2H), 3.09 (t, J = 7.8 Hz, 2H), 2.23 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9, 163.5, 156.7, 153.2, 148.8, 148.2, 143.4, 138.7, 138.1, 126.9, 115.1, 114.2, 108.4, 107.0, 102.8, 64.8, 63.2, 61.6, 56.7, 33.6, 32.4, 24.6; IR (film) ν_{max} 3437, 2947, 1657, 1521, 1470, 1430, 1310, 1250, 1116, 1092 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + Na^+] calcd for $C_{22}H_{24}NO_9Cl$, 504.1037; found, 504.1022.

2,3,6-Trimethoxy-5-nitrophenethyl-3-chloro-4,6-dihydroxy-2-(3-oxopropyl)benzoate (117a). Compound **116a** (226 mg, 0.47 mmol) was dissolved in dioxane (3 mL) and water (1 mL) before OsO_4 (4 wt. % in water, 3 mg, 0.01 mmol) and $NaIO_4$ (377 mg, 1.76 mmol) were added at room temperature. The slurry was stirred for 2 h and filtered through a plug of Celite. Water (3 mL) and EtOAc (15 mL) were added to the eluent, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (15 mL) and saturated aqueous NaCl (15 mL) and dried (Na_2SO_4). The residue was purified via chromatography (SiO_2, 20% EtOAc in hexanes) to afford **117a** (213 mg, 94%) as a yellow solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.38 (s, 1H), 9.76 (s, 1H), 7.46 (s, 1H), 6.59 (s, 1H), 6.06 (s, 1H), 4.50 (t, J = 7.2 Hz, 2H), 3.98(s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.31 (t,

J = 7.6 Hz, 2H), 3.14 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.6, 170.4, 163.4, 157.0, 153.1, 148.8, 148.0, 141.9, 138.6, 126.8, 114.3, 108.4, 107.0, 103.3, 65.0, 63.2, 61.6, 56.7, 43.4, 25.7, 24.5; IR (film) ν_{max} 3400, 2945, 2849, 1720, 1658, 1520, 1481, 1425, 1310, 1250, 1115, 1092 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + NH_4^+] calcd for $C_{21}H_{26}N_2O_{10}Cl$, 501.1276; found, 501.1269.

3-(2-Chloro-3,5-dihydroxy-6-((2,3,6-trimethoxy-5-nitrophenethoxy)carbonyl)phenyl)propanoic Acid (118a). Compound **117a** was redissolved in *t*-BuOH (1.2 mL), 2-methyl-2-butene (1.2 mL), and water (0.2 mL) before $NaClO_2$ (31 mg, 0.34 mmol) and NaH_2PO_4 (63 mg, 0.45 mmol) were added to the solution at room temperature. The mixture was stirred for 30 min at room temperature. Saturated aqueous NaH_2PO_4 (2 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with H_2O (10 mL) and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 40% EtOAc in hexanes) to afford **118a** (107 mg, 95%) as a pale yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46 (s, 1H), 6.60 (s, 1H), 4.56 (t, J = 7.1 Hz, 2H), 3.98 (s, 3H), 3.92 (s, 3H), 3.88 (s, 3H), 3.33 (t, J = 8.1 Hz, 2H), 3.20 (t, J = 7.1 Hz, 2H), 2.56 (t, J = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.3, 170.0, 163.1, 156.1, 152.7, 148.3, 147.6, 140.8, 138.2, 126.4, 113.8, 108.0, 106.9, 102.9, 64.6, 62.7, 61.0, 56.2, 32.8, 27.9, 24.0; IR (film) ν_{max} 3500, 2945, 1708, 1659, 1521, 1483, 1309, 1298, 1200, 1114, 1092 1027 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + Na^+] calcd for $C_{21}H_{22}NO_{11}ClNa$, 522.0779; found, 522.0795.

3-(2-((3-Amino-2,5,6-trimethoxyphenethoxy)carbonyl)-6-chloro-3,5-dihydroxyphenyl)propanoic Acid (119a). Compound **118a** (100 mg, 0.20 mmol) was dissolved in EtOH (2.0 mL) before 10% palladium on carbon (10 mg) was added. The mixture was purged with argon before H_2 gas was added. The heterogeneous mixture was stirred for 3 h and then filtered through a plug of Celite. The solvent was removed under reduced pressure, and the residue was purified via chromatography (SiO_2, 60% EtOAc in hexanes) to afford **119a** (86 mg, 92%) as a colorless solid: ^{1}H NMR (CD_3OD, 400 MHz) δ 6.44 (s, 1H), 6.40 (s, 1H), 4.48 (t, J = 7.7 Hz, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.72 (s, 3H), 3.24 (t, J = 7.9 Hz, 2H), 3.11 (t, J = 7.7 Hz, 2H), 2.50 (t, J = 8.0 Hz, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 169.8 (2), 160.4, 157.5, 149.5, 140.8, 139.4, 139.3, 136.3, 123.5, 113.5, 107.8, 101.7, 100.0, 64.7, 60.0, 59.1, 54.9, 33.2, 27.6, 23.6; IR (film) ν_{max} 3480, 2943, 1708, 1654, 1605, 1492, 1315, 1250, 1119, 1089, 1020, 1007 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + H^+] calcd for $C_{21}H_{25}NO_9Cl$, 470.1218; found, 470.1220.

Desmethyltrimethoxy Radanamycin (120a). Compound **119a** (82 mg, 0.18 mmol) was dissolved in DMF (10 mL) before the slow addition to a mixture of HATU (133 mg, 0.35 mmol), HOBt (47 mg, 0.35 mmol), and diisopropyl ethylamine (90 uL, 0.7 mmol) in DMF (190 mL) over 4 h. Upon addition, the solution was heated at 70 °C for 15 h. The solvent was removed by distillation at reduced pressure. EtOAC (30 mL) was added to the residue, and the resulting solution was washed with saturated aqueous NH_4Cl (3 × 5 mL), H_2O (5 mL), and saturated aqueous NaCl (5 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 20% hexanes in EtOAc) to afford **120a** (68 mg, 84% yield) as a colorless solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.98 (s, 1H), 6.91 (s, 1H), 6.68 (s, 1H), 6.53 (s, 1H), 6.18 (s, 1H), 4.64 (m, 1H), 4.39 (m, 1H), 4.10 (m, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 3.69 (s, 3H), 3.37 (t, J = 5.2 Hz, 1H), 3.10 (m, 2H), 2.12 (m, 1H), 1.96 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.3, 172.0, 164.0, 156.2, 150.5, 149.5, 146.8, 142.2, 125.7, 125.5, 114.0, 110.6, 106.6, 102.8, 67.3, 61.1(2), 56.0, 33.8, 28.0, 23.0; IR (film) ν_{max} 3000, 2850, 1645, 1635, 1487, 1456, 1320, 1236, 1100, 1080 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + H^+] calcd for $C_{21}H_{23}NO_8Cl$, 452.1112; found, 452.1106.

Ethyl 3-(2-Methoxy-3,6-bis(methoxymethoxy)phenyl)acrylate (112). To the solution of NaH (1.4 g, 34.3 mmol) in toluene (150 mL) was added triethyl phosphonoacetate (3.75 mL, 18.7 mmol) at 0 °C, and the solution was warmed to room temperature. After 15 min, **25** (4.0 g, 15.6 mmol) was added to the mixture and stirred overnight. A saturated aqueous NH_4Cl (20 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H_2O (60 mL) and saturated aqueous NaCl (80 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 10% EtOAc in hexanes) to afford **112** (4.6 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.02 (d, J = 9.1 Hz, 1H), 7.10 (d, J = 9.1 Hz, 1H), 6.92 (m, 2H), 5.21 (s, 2H), 5.17 (s, 2H), 4.28 (q, J = 7.1 Hz, 4H), 3.87 (s, 3H), 3.53 (s, 3H), 3.49 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$ 100 MHz) δ 168.6, 152.5, 150.9, 145.6, 135.9, 122.7, 119.7, 119.4, 110.5, 96.3, 95.3, 61.3, 60.7, 56.7, 56.6, 14.8; IR (film) ν_{max} 3398, 3300, 2980, 1521, 1470, 1151, 1051 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + NH_4^+] calcd for $C_{16}H_{26}NO_7$, 344.1709; found, 344.1720.

3-(2-Methoxy-3,6-bis(methoxymethoxy)phenyl)propan-1-ol (30). Compound **112** (5.8 g, 17.8 mmol) was dissolved in in EtOAc (100 mL) before 10% palladium on carbon (0.58 mg) was added. The mixture was purged with argon before H_2 gas was added. The heterogeneous mixture was stirred for 5 h and then filtered through a plug of Celite. The eluent was concentrated, and the residue was dissolved in CH_2Cl_2 (80 mL). To the mixture was slowly added DIBAL (32.3 mL, 32.2 mmol) at 0 °C, and the mixture was stirred for 1 h. Then, a saturated aqueous Rochele salts (30 mL) was deed at 0 °C and stirred overnight. The aqueous layer was extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were washed with H_2O (40 mL) and saturated aqueous NaCl (40 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 10% EtOAc in hexanes) to afford **30** (4.1 g, 80%, 2 steps) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.95 (d, J = 9.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 1H), 5.17 (s, 2H), 5.16 (s, 2H), 3.85 (s, 3H), 3.54 (t, J = 5.8 Hz, 2H), 3.45 (s, 3H), 3.44 (s, 3H), 2.80 (t, J = 6.9 Hz, 2H), 2.54 (br s, 1H), 1.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.2, 149.2, 145.5, 125.2, 115.3, 110.0, 96.1, 95.4, 61.7, 61.5, 56.6, 56.5, 32.4, 20.0; IR (film) ν_{max} 3420, 2939, 2361, 1593, 1488, 1256, 1103 cm^{-1}; HRMS (TOF-ES$^+$) m/z: [M + Na^+] calcd for $C_{14}H_{22}O_6Na$, 309.1314; found, 309.1325.

4-(2-Methoxy-3,6-bis(methoxymethoxy)phenyl)butan-1-ol (113). To a solution of $BrPPh_3(CH_2)_3OCH_2Ph$ (14.73 g, 30 mmol) in THF (200 mL) was added $LiN(SiMe_3)_2$ (25 mL, 1.2 M, 30 mmol) at −78 °C. After 5 min, the mixture was warmed to 0 °C and stirred until the solution became clear. Then, the mixture was cooled to −78 °C before **25** (5.12 g, 20 mmol) was added. The mixture was warmed to rt and stirred for an additional 10 h. A saturated aqueous NH_4Cl (50 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H_2O (60 mL) and saturated aqueous NaCl (80 mL) and dried (Na_2SO_4), filtered, and concentrated. The crude product was filtered through a plug of silica gel (10% EtOAC in hexanes). The eluent was concentrated and dissolved in EtOAc (15 mL) before 10% palladium on carbon (0.7 g) was added. The mixture was purged with argon before H_2 gas was added. The heterogeneous mixture was stirred for 5 h and then filtered through a plug of Celite. The residue was purified via chromatography (SiO_2, 30% EtOAc in hexanes) to afford **113** (4.9 g, 83%, 2 steps) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (d, J = 9.1 Hz, 1H), 6.78 (d, J = 8.9 Hz, 1H), 5.15 (s, 4H), 3.85 (s, 3H), 3.67 (t, J = 5.6 Hz, 2H), 3.54 (s, 3H), 3.52 (s, 3H), 2.68 (t, J = 6.2 Hz, 2H), 2.05 (br s, 1H), 1.63 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.2, 149.2, 145.6, 126.7, 115.1, 109.9, 96.1, 95.3, 63.2, 61.4, 56.5, 56.4, 32.9, 26.4, 24.0; IR (film) ν_{max} 3438, 2935, 1593, 1485, 1404, 1248, 1153, 1070 cm^{-1}; HRMS (TOF-ES$^+$) *m/z*: [M + Na^+] calcd for $C_{15}H_{24}O_6Na$, 323.1471; found, 323.1464.

4-(2-Methoxy-3,6-bis(methoxymethoxy)-5-nitrophenyl)butan-1-ol (114). To a mixture of **113** (1.1 g, 3.5 mmol) and NH_4NO_3 (294 mg, 3.68 mmol) in THF (30 mL) was slowly added trifluoroacetic anhydride (1.94 mL, 14 mmol), and the mixture was

stirred for 1 h at room temperature. A solution of LiOH (3.2 g, 76 mmol) in MeOH (10 mL) and H_2O (10 mL) was added to the mixture and stirred for 10 min before a saturated aqueous $NaHCO_3$ was added at room temperature. The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with H_2O (60 mL) and saturated aqueous NaCl (80 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 30% EtOAc in hexanes) to afford **114** (1.0 g, 85%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (s, 1H), 5.20 (s, 2H), 5.00 (s, 2H), 3.92 (s, 3H), 3.67 (t, J = 5.2 Hz, 2H), 3.55 (s, 3H), 3.50 (s, 3H), 2.70 (t, J = 6.2 Hz, 2H), 1.81 (br s, 1H), 1.62 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.2, 146.6, 145.6, 140.0, 133.2, 111.2, 102.1, 95.8, 62.8, 61.6, 58.1, 56.9, 33.0, 26.5, 25.0; IR (film) ν_{max} 3356, 2939, 2359, 1524, 1340, 1290, 1240, 1155, 1057, 1022 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + NH_4] calcd for 363.1767, $C_{15}H_{23}NO_8Na$; found, 363.1774.

Methyl 4,6-Bis(*tert*-butyldimethylsilyloxy)-3-chloro-2-(pent-4-enyl)benzoate (110a). To a solution of **109** (4.6 g, 10.3 mmol) in THF (25 mL) was added lithium diisopropylamide (6.7 mL, 2 M in THF/Heptanes, 13.4 mmol) at −40 °C. After 10 min, to the mixture was slowly added 4-bromo-1-butene (2.2 g, 16.5 mmol), and the mixture was stirred for 1 h at −40 °C before a saturated aqueous NH_4Cl (5 mL) solution was added. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with H_2O (20 mL) and saturated aqueous NaCl (20 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 2% EtOAc in hexanes) to afford **110a** (4.4 g, 86%) as a colorless solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.31 (s, 1H), 5.80 (m, 1H), 4.98 (m, 2H), 3.86 (s, 3H), 2.63 (m, 2H), 2.11 (m, 2H), 1.66 (m, 2H), 1.05 (s, 9H), 0.98 (s, 9H), 0.24 (s, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.6, 153.2, 151.6, 140.1, 138.7, 121.5, 119.0, 115.2, 109.5, 52.5, 34.2, 31.9, 29.0, 26.0 (2), 25.9, 18.8, 18.4, −3.97 (2), −4.01 (2); IR (film) ν_{max} 2950, 2932, 2858, 1735, 1585, 1460, 1420, 1407, 1356, 1254, 1200, 1107, 1041, 843, 783 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + H$^+$] calcd for $C_{25}H_{44}O_4ClSi_2$, 499.2467; found, 499.2444.

3-Chloro-4,6-dihydroxy-2-(pent-4-enyl)benzoic Acid (111a). To a solution of **110a** (5.5 g, 11 mmol) in THF (15 mL), MeOH (5 mL), and H_2O (5 mL) was added LiOH (1.3 g, 32 mmol) at room temperature. Affer 1 h, the solvent was removed and EtOAc (100 mL) was added. The solution was washed with H_2O (2 × 50 mL) and saturated aqueous NaCl (50 mL) and dried (Na_2SO_4), filtered, and concentrated. The crude product was filtered through a plug of silica gel (15% EtOAC in hexanes). The eluent was concentrated and dissolved in HMPA (5 mL) before freshly prepared lithium 1-propanethiolate (48 mL, 24 mmol, 0.5 M in HMPA) was added at room temperature. After 2.5 h, aqueous HCl (20 mL, 4 M) was added at 0 °C and stirred for 30 min. The mixture was diluted with EtOAc (200 mL), and the organic phase was washed with aqueous HCl (3 × 20 mL, 2 M) and H_2O (3 × 20 mL). The solvent was removed before an aqueous solution of NaOH (30 mL, 2M) was added at 0 °C. The mixture was washed with EtOAc (2 × 20 mL) and treated with aqueous HCl (2 M) until pH = 3. The acidified aqueous phase was extracted with EtOAc (2 × 80 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated to afford **111a** (2.3 g, 82%, 2 steps) as a colorless solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.50 (s, 1H), 5.76 (m, 1H), 4.92 (m, 2H), 3.08 (m, 2H), 2.11 (m, 2H), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 164.3, 157.1, 145.3, 138.2, 115.0, 114.2, 105.0, 102.4, 34.0, 32.4, 28.7; IR (film) ν_{max} 3375, 2935, 1635, 1600, 1456, 1424, 1265, 1165 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + H$^+$] calcd for $C_{12}H_{14}O_4Cl$, 257.0581; found, 257.0571.

MOM-Protected Desmethyl Radanamycin (120c). Compound **118c** (61 mg, 0.11 mmol) was dissolved in EtOH (2.0 mL) before 10% palladium on carbon (12 mg) was added. The mixture was purged with argon before H_2 gas was added. The heterogeneous mixture was stirred for 3 h and then filtered through a plug of Celite. The eluent was concentrated, and the residue was redissolved in DMF (10 mL) before the slow addition to a mixture of HATU (125 mg, 0.33 mmol), HOBt (45 mg, 0.33 mmol), and diisopropyl ethylamine (0.17 mL, 0.99 mmol) in DMF (100 mL) over 4 h. Upon addition, the solution was heated at 70 °C for 15 h. The solvent was removed by distillation at reduced pressure. EtOAC (20 mL) was added to the residue, and the resulting solution was washed with saturated aqueous NH_4Cl (3 × 5 mL), H_2O (5 mL), and saturated aqueous NaCl (5 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 20% hexanes in EtOAc) to afford **120c** (39 mg, 77% yield, 2 steps) as a colorless solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.0 (s, 1H), 6.97 (s, 1H), 6.93 (s, 1H), 6.54 (s, 1H), 6.37 (br s, 1H), 5.16 (dd, J = 6.6, 24.1 Hz, 2H), 4.89 (dd, J = 6.6, 24.1 Hz, 2H), 4.63 (m, 1H), 4.42 (m, 1H), 4.06 (m, 1H), 3.98 (s, 3H), 3.61 (s, 3H), 3.53 (s, 3H), 3.36 (m, 1H), 3.07 (m, 2H), 2.10 (m, 1H), 1.92 (m, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.2, 171.9, 163.9, 156.3, 148.7, 147.8, 147.4, 142.1, 126.5, 126.1, 114.8, 114.1, 105.5, 102.8, 100.2, 95.4, 67.2, 61.4, 58.1, 56.5, 34.0, 27.9, 23.5; IR (film) ν_{max} 3133, 2929, 1651, 1597, 1487, 1128, 1018 cm^{-1}; HRMS (TOF-ES$^+$) m/z: [M + H$^+$] calcd for $C_{23}H_{27}NO_{10}Cl$, 512.1323; found, 512.1338.

Desmethyl Radanamycin (121c). Compound **120c** (40 mg, 0.078 mmol) was dissolved in CH_3CN (1.2 mL) and CH_2Cl_2 (1.2 mL) before NaI (88 mg, 0.78 mmol) and TMSCl (0.104 mL, 0.78 mmol) were added at room temperature. Upon addition, the solution turned cloudy and yellow. After 20 min, a saturated aqueous solution of $Na_2S_2O_4$ (2 mL) was added to the mixture and stirried for 10 min. The aqueous phase was extracted with EtOAc (3 × 5 mL), and the combined organic layers were washed with H_2O (2 × 5 mL) and saturated aqueous NaCl (5 mL) and dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 10% MeOH in CH_2Cl_2) to afford **121c** (29 mg, 89% yield) as a colorless solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.91 (s, 1H), 7.85 (br s, 1H), 6.58 (s, 1H), 6.45 (s, 1H), 4.72 (m, 1H), 4.57 (m, 1H), 3.91 (s, 3H), 3.51 (m, 2H), 3.10 (m, 1H), 3.00 (m, 1H), 2.07 (m, 1H), 1.99 (m, 1H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 175.1, 170.3, 159.0, 156.9, 147.4, 146.9, 144.2, 140.9, 122.2, 120.5, 115.2, 113.6, 112.8, 103.4, 66.2, 61.0, 34.6, 28.5, 24.2; IR (film) ν_{max} 3540, 3306, 3049, 1702, 1643, 1444, 1379, 1236, 1172 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + H$^+$] calcd for $C_{19}H_{19}NO_8Cl$, 424.0799; found, 424.0799.

Desmethyl Radanamycin Quinone (122c). To a solution of **121c** (42 mg, 0.1 mmol) in EtOAc (2 mL) and MeOH (0.4 mL) was added 10% palladium on carbon (42 mg), and the solution was stirred in open air at room temperature. After 2 h, the mixture was filtered through a plug of Celite. The eluent was concentrated, and the residue was purified via chromatography (SiO_2, 4% MeOH in CH_2Cl_2) to afford **122c** (29 mg, 70% yield) as a yellow solid: ^{1}H NMR (acetone-d_6, 500 MHz) δ 9.50 (s, 1H), 9.31 (br s, 1H), 8.83 (s, 1H), 6.56 (s, 1H), 6.25 (s, 1H), 4.88 (m, 2H), 4.20 (s, 3H), 3.35 (m, 3H), 3.05 (m, 1H), 2.28 (m, 2H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 185.1, 183.9, 174.3, 171.3, 161.5, 158.0, 157.0, 145.7, 141.6, 127.3, 119.4, 114.0, 110.0, 103.3, 67.3, 61.7, 38.5, 28.8, 22.8; IR (film) ν_{max} 3560, 3000, 2910, 2802, 1710, 1655, 1600, 1400, 1211, 1118, 1056 cm^{-1}; HRMS (TOF-ES$^+$) *m*/*z*: [M + H$^+$] calcd for $C_{19}H_{17}NO_8Cl$, 422.0643; found, 422.0646.

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Supporting Information Available: Full experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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