JOC Article

Total Synthesis of (+)-Geldanamycin and (−)-*o*-Quinogeldanamycin: Asymmetric Glycolate Aldol Reactions and Biological Evaluation

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The total synthesis of (+)-geldanamycin (GA), following a linear route, has been completed using a demethylative quinone-forming reaction as the last step. Key steps include the use of two new asymmetric boron glycolate aldol reactions. To set the *anti*-C11,12 hydroxymethoxy functionality, (*S*,*S*)-5,6-bis-4-methoxyphenyldioxanone **8** was used. Methylglycolate derived from norephedrine **5** set the C6,7 methoxyurethane stereochemistry. The quinone formation step using nitric acid gave the non-natural *o*-quino-GA product **55** 10:1 over geldanamycin. Other known oxidants gave an unusual azaquinone product **49**. *o*-Quino-GA **55** binds Hsp90 with good affinity but is less cytotoxic compared to GA.

While macbecin I and herbimycin A have received considerable synthetic attention, including four total syntheses,[1] the closely related ansamycin antitumor antibiotic geldanamycin (GA) has only recently succumbed to total synthesis.[2] It was isolated (*Streptomyces hygroscopicus* var. *geldanus)* in 1970 by workers at Upjohn, and the structure was determined by Rinehart and co-workers shortly thereafter.[3] These compounds possess various biological activities, including great potential as therapeutic lead compounds for new anticancer agents. Various semisynthetic analogues have been made and tested.[4] Among these, the phase-I clinical candidate 17-allylamino-GA is the most prominent. Ironically, geldanamycin, with the least synthetic attention, was discovered first and is the most potent member of this class. It shows broad activity with a unique profile of action within the NCI 60 cell-line panel with an average ED_{50} value of 180 nM.[5]

Lack of initial interest in this target may be attributed to two factors: One, only recently has its cellular target been identified, and two, it presents distinct and significant synthetic challenges compared to the other ansamycins. Neckers, through the use of a GA-affinity protocol, demonstrated that geldanamycin binds to the chaperone heat shock protein 90 (Hsp90).[6] Previously, GA was known to greatly lower cellular levels of various oncogenic tyrosine kinases, including v-Src, Bcr/Abl, and ErbB-2,[7] proteins that rely on Hsp90 for proper folding and stability. GA does not effect cellular levels of the serine/threonine kinases PKA or PKC. In addition, X-ray crystal structures have recently been reported for the Hsp90-GA complex.[8] GA binds to the ATP binding site in a C-shaped conformation distinct from its free, solution conformation.

In light of the recently disclosed biological properties of GA together with the challenge posed by its structure, the development of a total synthesis was clearly warranted. In this paper we outline the details of this route, which establishes the absolute stereochemistry, highlights two new glycolate aldol reactions, uncovers the challenge of *p*-quinone formation, and provides access to three new biologically active analogues (Scheme 1).

Unlike the related ansamycins, geldanamycin possesses a methoxyl at C17 on the quinone that requires the use of a pentasubstituted benzene precursor in order

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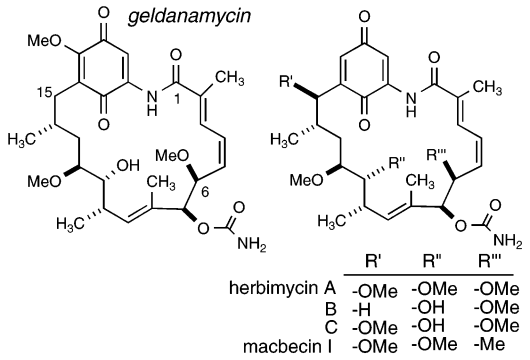
SCHEME 1



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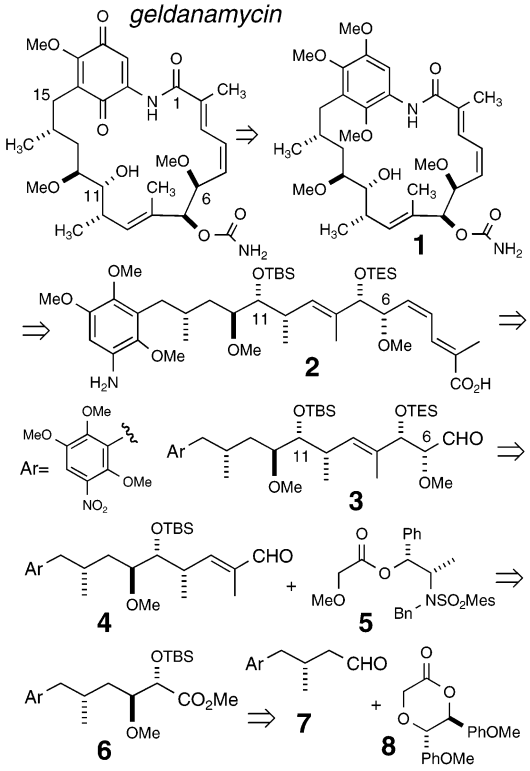
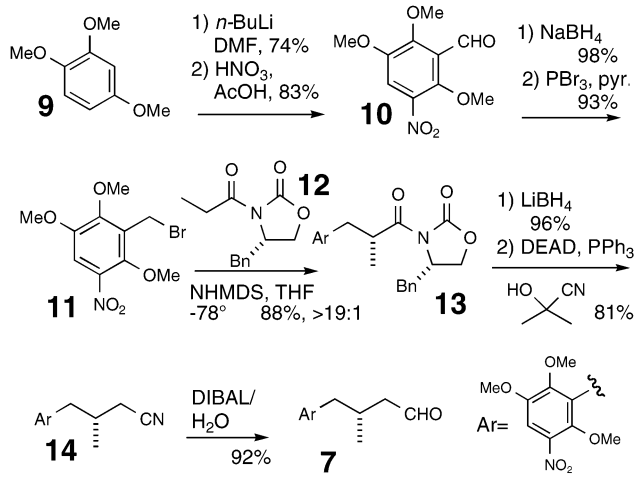


to ensure proper formation of the desired *p*-quinone (Scheme 2). In accord with previous macbecin and herbimycin studies where 1,4-dimethoxy compounds were used with success[1] and with results from other trimethoxybenzene substrates,[9] it was anticipated that oxidative removal of the 1,4-disposed methoxyls, as with intermediate **1**, would generate the needed *p*-quinone with high selectivity. Unforeseen conformational and electronic issues would, only at the very end of route, reveal the problem of this assumption. The lack of a hydroxyl at C15, together with the presence of the C17-methoxyl, prevents the use of an aldol reaction to form the C14−15 bond, as previously employed in the macbecin and herbimycin syntheses. In addition, C11 possesses a hydroxyl, not a methoxyl. Because of this, the route to GA needs to address the *anti*-C11,12 hydroxy−methoxy functionality in a way that can differentiate between these two groups. The *seco*-amino acid **2** reveals the dense arrangement of functionality. A suitable position for retrosynthetic cleavage at a midpoint for planning a convergent approach is problematic. The six stereocenters and the trisubstituted olefin crowd this region. The C7 TES (triethylsilyl) and C11 TBS (*tert*-butyldimethyl) silyl ethers were chosen to allow for the selective introduction of the urethane group. The aniline moiety would be masked throughout as an aryl nitro group. In this linear approach, aldehyde **3** would be used to form the *E*,*Z*-diene carboxylic acid. Enal **4** would undergo an asymmetric *syn*-glycolate aldol reaction with the newly reported norephedrine ester **5** to set the stereochemistry at C6 and C7.[10] While new approaches to the C8,9 olefin and C10-methyl region were attempted, the previously employed asymmetric hydroboration route was followed.[1a,d] Intermediate **6** was made using an asymmetric *anti*-glycolate aldol reaction with the newly reported bisaryldioxanone **8** reacting with aldehyde **7**.[11]

SCHEME 3



Following the lead of Gilman who reported that 1,2,4-trimethoxybenezene **9** could be selectively metalated at the 3 position,[12] treatment with *n*-butyllithium, followed by addition of DMF, lead to the formation of the substituted benzaldehyde (Scheme 3). Nitric acid in warm acetic acid then generated nitrobenzene **10**. Reduction and treatment with phosphorus tribromide gave the stable, crystalline benzyl bromide **11**. The C14 methyl stereocenter was set using an asymmetric Evans alkylation[13] upon deprotonation of (*S*)-**12** with NHMDS followed by addition of **11** (1.1 equiv) to give the product **13** in high yield and excellent selectivity. The primary alcohol was formed with lithium borohydride and Mit-

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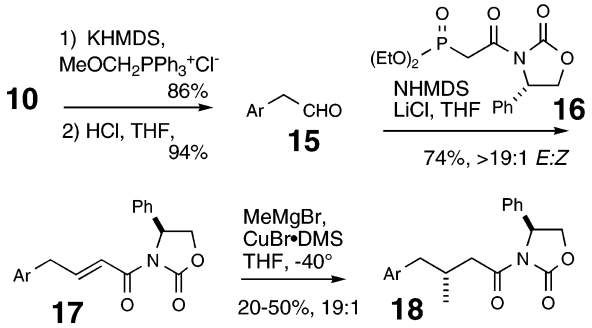
SCHEME 4

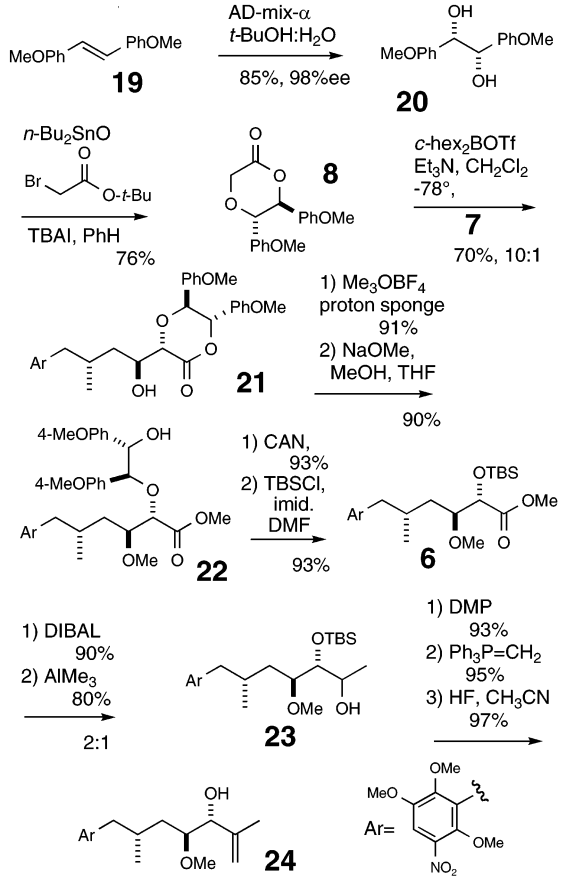


sunobu conditions using acetone cyanohydrin[14] were employed to directly give cyanide **14**. Two equivalents of DIBAL used at −78 °C followed by warming and addition of pH 7 buffer gave the key aldehyde **7** in high yield.

This route was not the initial choice to produce **7**. An asymmetric conjugate addition approach was also pursued by homologating aldehyde **10** with a methoxymethyl Wittig reagent to give the arylacetaldehyde **15** (Scheme 4).[15] Various attempts were made to then access the unsaturated acyloxazolidinone **17** using phosphonium and phosphonate derivatives with **15**. Finally, it was found that use of phosphonate **16** reacted with NHMDS in the presence of lithium chloride gave **17** with good reactivity and high selectivity.[16] Use of DBU in place of NHMDS gave only a 39% yield with 9:1 selectivity.[17] Other base and solvent combinations were also less effective. While the selectivity of the methylcuprate was found to be high in this case, typically >19:1, the yields rarely approached 50%. All variations attempted gave uniformly low yields.[18] It was apparent that under these conditions, nucleophilic demethylation of the benzene functionality was occurring leading to decomposition in this case. The success of the alkylation route ended the investigation of this route.

With aldehyde **7** in hand, a scaleable route to the *anti*-aldol step was pursued (Scheme 5). On a large scale, (*E*)-4,4′-dimethoxystilbene **19** was dihydroxylated under Sharpless conditions with the components of AD-mix-α added individually including methylsulfonamide to give (*S*,*S*)-diol **20**.[19] Batches as large as 10 g were transformed with reproducible results reacted at 0 °C. Diol **20** was treated with di-*n*-butyltin oxide in benzene at reflux using a Dean−Stark trap to remove water.[20] The resulting tin acetal was transformed with *tert*-butyl bromoacetate in the presence of tetra-*n*-butylammonium iodide to give dioxanone **8**. Following the reported method, **8** was treated with dicyclohexylboron triflate with added triethylamine to give the boron enolate.[11] Aldehyde **7** (1.2 equiv) was then reacted to give the (*S*,*S*)-*anti*-aldol adduct **21** in 70% yield with 10:1 selectivity. The minor product was assumed to be the (*R*,*R*)-*anti* product. The enolate of **8** is locked as the *E*-isomer and attack of the aldehyde is constrained to a closed Zimmerman−Traxler chair arrangement on the *si*-enolate face away from the C-5 methoxyphenyl. The alcohol was converted to the methyl ether and treated with catalytic sodium methoxide to give ester **22**. The benzyl ether was then cleaved using ceric ammonium nitrate and the TBS ether was generated to give the key intermediate **6**.

SCHEME 5



At this point, various routes to install the C8−10 functionality were considered. Ultimately, an asymmetric hydroboration approach proved successful as pioneered by Tatsuda with herbimycin A and used recently in Panek's approach to macbecin I.[1a,d] Other approaches explored at this key juncture will also be described briefly. To prepare for the hydroboration route, ester **6** was converted to the aldehyde and reacted with trimethylaluminum to give a mixture of alcohols **23** (Scheme 5). Oxidation with Dess−Martin periodinane and treatment with HF gave allyl alcohol **24**.[21] Excess borane•THF at −10 °C was used followed by peroxide to give a 5:1 mixture of *syn*- and *anti*-**25** diol products (Scheme 6). All

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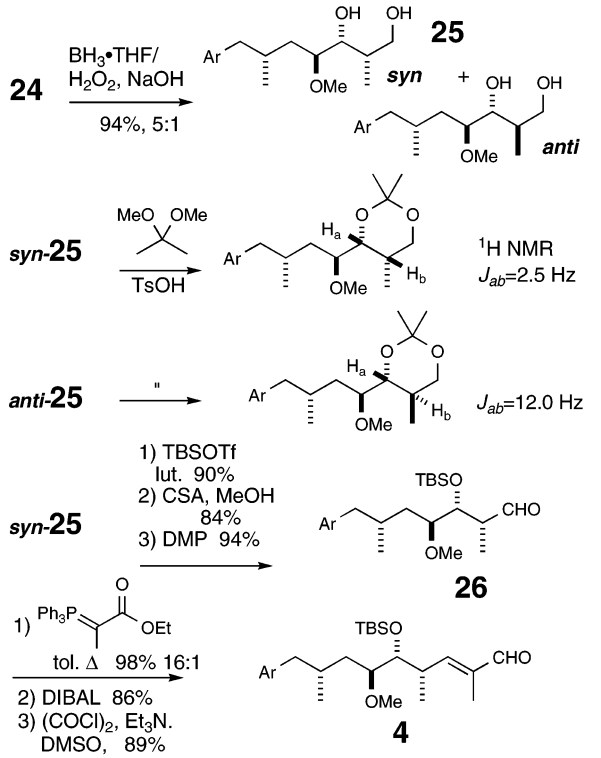
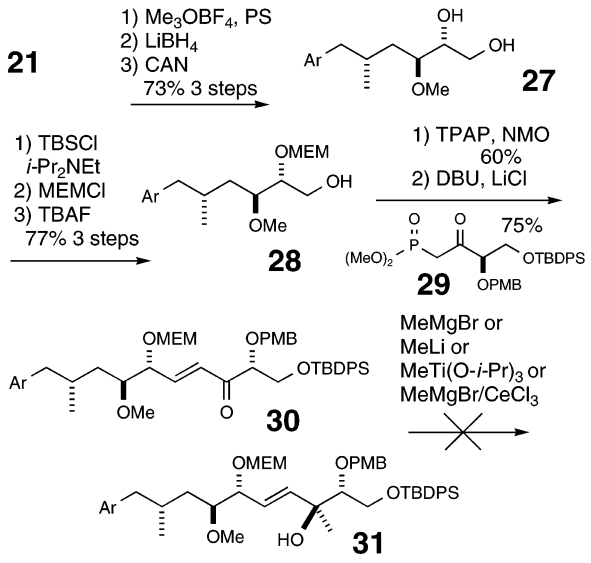
SCHEME 6



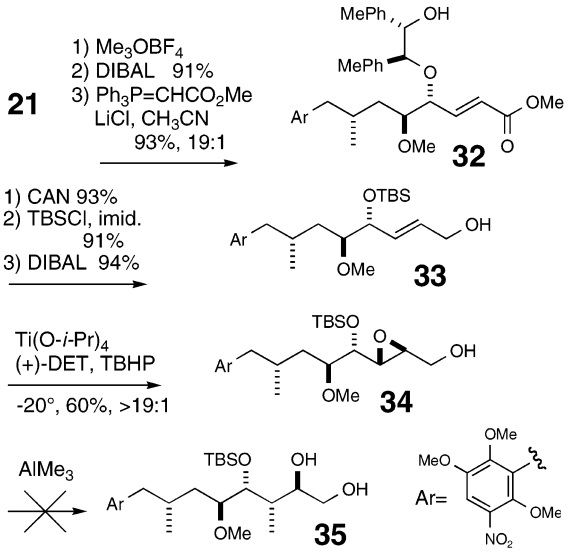
attempts to improve the selectivity failed. Fortunately, the yield was high and the minor, undesired anti isomer, due to a favorable R_f difference, was easily removed by chromatography. Evans and Burgess have shown that *syn*-diols from alkenes of this type can be accessed using rhodium-catalyzed hydroboration.[22] Panek's macbecin I approach found that the anti isomer was major under these conditions.[1d] While the origin of the asymmetric induction in this case remains unclear, Houk has proposed a transition-state model for the uncatalyzed reaction.[23] Conformations with the hydroxyl adopting an inside position over the outside position are favored theoretically, but only slightly by ~0.1 kcal/mol. In view of these ambiguities, a structure proof on both isomers of **25** was performed. The acetonides of the separated isomers were formed and indicated methine proton couplings were observed. The syn isomer J_{ab} was 2.5 Hz consistent with an axial−equatorial disposition and the anti isomer acetonide gave a diaxial 12.0 Hz value. Confident in the assignment, the major *syn*-**25** isomer was protected, with TBS triflate, followed by camphor sulfonic acid to remove the primary silyl ether and oxidized to access aldehyde **26**. Stabilized Wittig treatment gave the unsaturated ester in quantitative yield with high *E* selectivity. DIBAL reduction followed by Swern oxidation then gave enal **4**.

A more convergent approach to GA using an enone intermediate was explored (Scheme 7). The aldol adduct **21** was converted to the 1,2-diol product **27** and protected

SCHEME 7



SCHEME 8



to give the methoxyethoxymethyl (MEM) ether **28**. Oxidation, followed by coupling with ketophosphonate **29**,[17] previously described,[24] gave enone **30** in good yield with complete *E* selectivity. Unfortunately, all attempts to add Mg or Li methylorganometallics gave rapid decomposition, or no reaction, as with titanium reagents. The desired alcohol **31**, allowing for conversion to the C8,9 olefin via allylic displacement, was not obtained in this case.

An alternative to the hydroboration route was also explored using an asymmetric Sharpless epoxidation (Scheme 8).[25] Adduct **21** was methylated and reduced to the lactol with DIBAL. Treatment with Wittig reagent in the presence of LiCl in acetonitrile gave a high yield of unsaturated ester **32**. CAN removal, protection, and reduction generated the allylic alcohol **33**. The standard conditions were then employed to produce the epoxide

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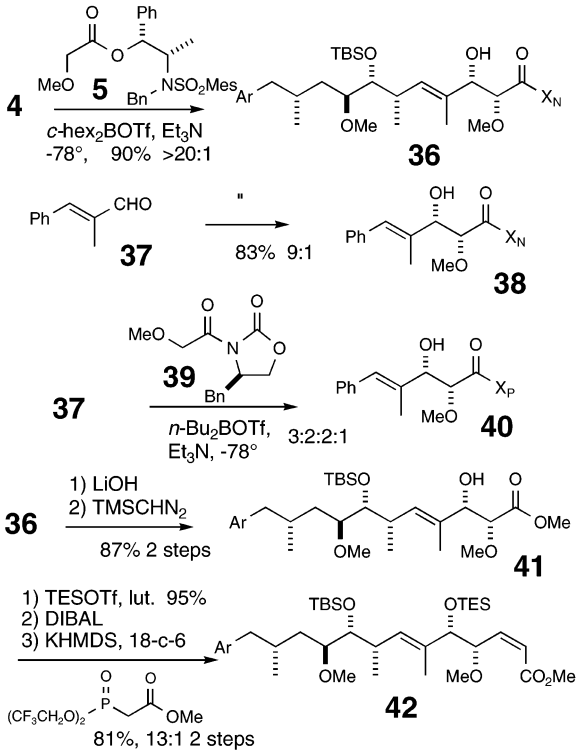
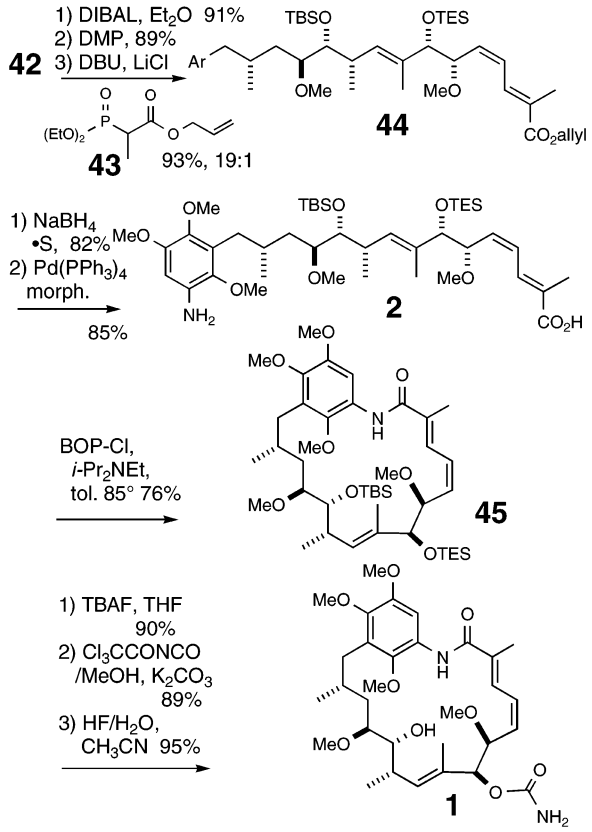
SCHEME 9



SCHEME 10



with high selectivity. Trimethylaluminum is well-known for its ability to open epoxy alcohols at the β-position to give 1,2-diol products.[26] However, all attempts using **34** at the standard temperature of 0 °C gave no reaction, and at higher temperatures only decomposition was seen. The γ-TBS ether was changed to smaller protecting groups, i.e., methyl, without success. No reaction and decomposition were again observed. The sensitivity of the substituted benzene substrate was a likely contributor to these challenges. After these detours, the linear hydroboration route was followed to completion.

From enal **4** to the lactam stage, the route to geldanamycin proceeded without complication. The newly developed (−)-norephedrine-derived glycolate **5** was reacted with boron triflate and triethylamine followed by addition of **4** to give syn adduct **36** in high yield and >20:1 selectivity (Scheme 9).[10] Two equivalents of the boron enolate of **5** were used to ensure complete conversion of **4**. The model substrate for this reaction was 2-methylcinnamaldehyde **37**. In this case, **5** reacted under similar conditions with this new auxiliary to give a 9:1 mixture of syn products. *syn*-Gylcolate aldol reactions are well-known using the Evans oxazolidinone auxiliary approach.[27] However, in this case, with substrate **37** reacted with oxazolidinone **39**, a mixture of all four possible **40** isomers, 3:2:2:1, was obtained. From this comparison, the utility of the norephedrine glycolate is demonstrated for α-branched enal substrates. Adduct **36** was converted to the methyl ester **41** using hydroxide followed by trimethylsilyldiazomethane.[28] The norephedrine alcohol was easily recovered by separation from the methyl ester at this point using chromatography. Attempts to obtain the alcohol after the carboxylic acid forming step using acid/base extraction proved futile. Protection as the TES ether at C7, followed by a half-reduction with DIBAL at −78 °C gave an aldehyde, which was reacted with the Still−Gennari hexafluorophosphonate in the presence of KHMDS and 18-c-6 to generate the (*Z*)-ester **42**.[29]

Reduction of (*Z*)-ester **42** initially proved problematic. When THF was used as solvent, even a great excess of DIBAL (10 equiv) gave only a <50% yield with the remainder being recovered **42**. Surprisingly, use of ether with DIBAL with only 2.5 equiv, gave a 91% yield of (*Z*)-allyl alcohol (Scheme 10). Use of CH_2Cl_2 as solvent for this step resulted in complete reduction and TES ether cleavage. Oxidation gave the enal, which was then reacted with allyl ester phosphonate **43** together with DBU and lithium chloride following the procedure of Roush.[30] The desired *E*,*Z* diene ester **44** was obtained in high yield and selectivity. Previously, the methyl ester of **44** was employed. Hydrolysis to the carboxylic acid from this intermediate was very slow and gave multiple products in that case. The arylnitro group was converted

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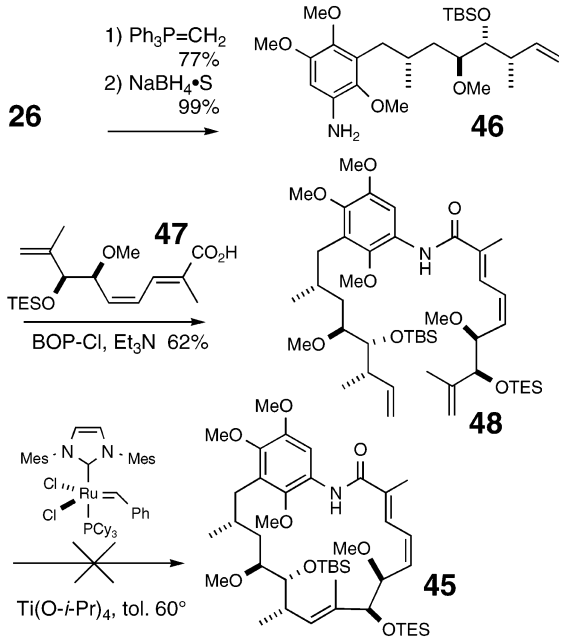
(30) Roush, W. R.; Sciotti, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7411.

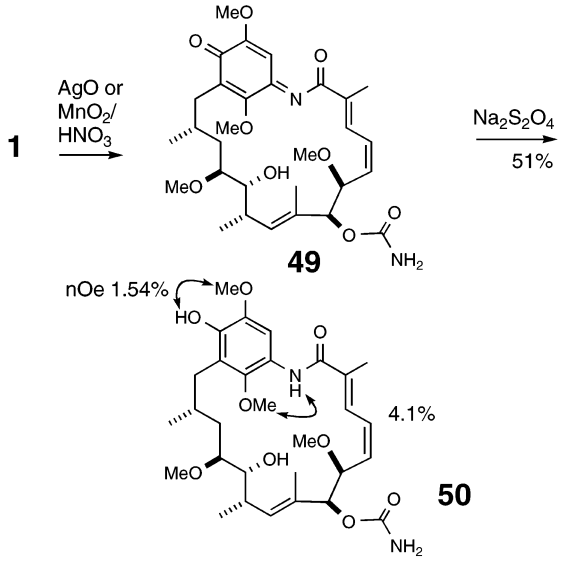
to the aniline at this point using the reagent described by Lalancette.[31] $NaBH_2S_3$ was formed with sodium borohydride reacted with 3 equiv of elemental sulfur as previously employed by Panek for macbecin I.[1d] The aniline from **44** was obtained in 82% yield. Removal of the allyl ester with palladium tetrakistriphenylphosphine and morpholine produced the key amino acid **2**. Dimedone, used in place of morpholine for this step, caused problems at the purification step. Use of BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride) with diisopropylethylamine in warm toluene (0.001 M) generated the macrolactam **45** in 76% isolated yield. At this point, various ansa ring conformations, including rotational isomers about the amide bond, complicated the NMR analysis. Changes in solvent and temperature gave only minimal improvement in the line broadening. Attention now focused on urethane introduction at C7. Tetra-*n*-butylammonium fluoride (TBAF, 2.1 equiv) in THF at 0 °C efficiently removed the TES ether. Previous ansamycin routes have used excess sodium cyanate with TFA to install the urethane with low yields (~50%) being typical.[1] We elected to employ trichloroacetyl isocyanate, following the procedure of Kocovsky.[32] The protected carbamate was initially formed and methanolysis with potassium carbonate gave the stable C7 urethane in 89% isolated yield. The standard conditions of TBAF or HF·pyridine failed to remove the C11 TBS ether even when used in excess. Finally, it was found that use of a 10:1 acetonitrile−aqueous HF (48%) mixture gave alcohol **1** in excellent yield. Flexible introduction of the urethane and removal of protecting groups boded well for exploration of the quinone formation.

To access lactam **45** in a more efficient, convergent manner, a ring-closing metathesis (RCM) strategy was explored.[33] While medium and large rings have found success with this approach, the combination of a ring of this size together with a trisubstituted olefin target possessing allylic substituents has not been reported. In view of the potential improvement in efficiency and the ready availability of the intermediates, the RCM route was attempted. Aldehyde **26** was converted to the terminal olefin−aniline **46** (Scheme 11). The carboxylic acid **47** was made using a series of analogous steps from isobutenal, including a syn glycolate aldol reaction and the allylester phosphonate reagent. Coupling under the BOP-Cl conditions gave the arylamide **48**. The Grubbs imidazolium benzylidene ruthenium catalyst, 10 mol %, was explored under a variety of conditions, including various solvents, concentrations, modes of addition, and additives, to no avail. Even added titanium tetraisopropoxide, to disfavor allylic oxygen coordination, in warm toluene[34] failed to give the desired lactam **45**.[35] The allylic C7 alcohol, following TES removal, was also explored as substrate and the diol from TBS removal from **47**. A

SCHEME 11



SCHEME 12



substrate involving a diphenylsiloxane tether between the C7 and C11 hydroxyls and intermolecular metathesis with **46** and **47** also failed.

Formation of the *p*-quinone from the trimethoxy precursor **1** is the final issue to be addressed. On the basis of the other ansamycin routes[1] and the various reports of successful oxidative demethylation reactions of others using more simple trimethoxybenzenes,[9] lactam **1** was anticipated to give GA directly (Scheme 12). Instead, using either silver or manganese oxide impregnated with nitric acid, the unusual aza-quinone variant **49** was rapidly obtained in 77 and 40% yields, respectively. Other known oxidants, including CAN in acetonitrile even at −10 °C, led only to decomposition in this case. The structure of this unique product **49** was confirmed by conversion to the corresponding dihydroazaquinone, lactam−phenol **50**, by treatment with sodium hydrosulfite.[36] The NOE enhancements of 1.54 and 4.1% were observed

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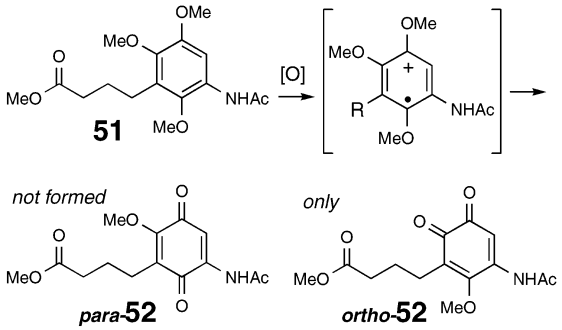
(35) A test substrate, *o*-allylphenyl acrylate, was employed under these conditions with success to verify the activity of the catalyst.

SCHEME 13

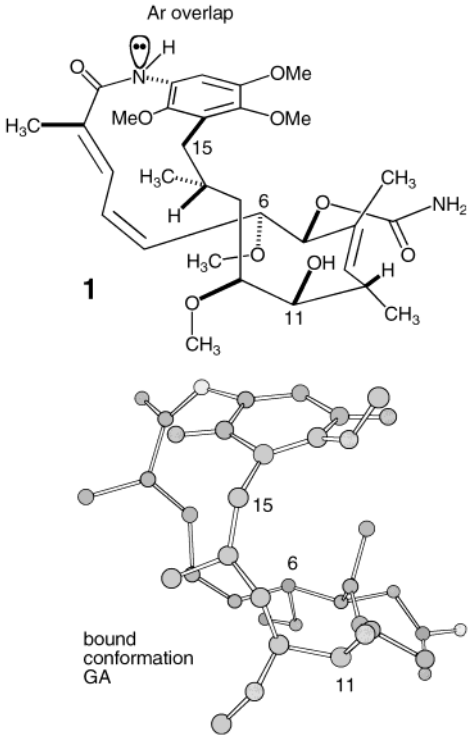


between the proton signals indicated. The oxidation was also explored with the C11 hydroxyl protected as the TBS ether. In this case, CAN and AgO/HNO_3 again produced the azaquinone as the sole product in only 10 min in good yield, 70−80%. No trace of the desired quinone was detected by TLC, NMR, or MS in crude material or in purified fractions.

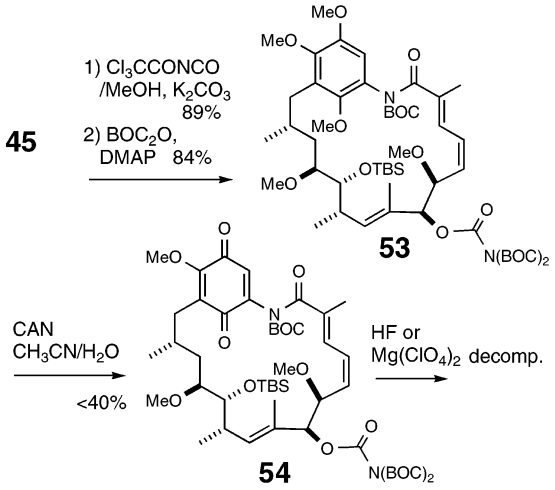
To anticipate possible complications, a model trimethoxybenzene amide **51**, made in a few steps from aldehyde **15**, was explored with various oxidants (Scheme 13). In this case, CAN[37] and AgO gave a quantitative yield of a quinone **52**. No trace of aza-quinone product was detected. Other oxidants including CoF_2,[38] $PhI(OAc)_2$, $PhI(OTf)_2$,[39] DDQ, and CrO_3 gave little or no product. Initially, through comparison of the NMR data to related compounds, we were confident that the quinone obtained in this case was the *p*-quinone. Only after considerable effort was a crystal suitable for X-ray analysis obtained for **52**.[40] Surprisingly, this unambiguous result confirmed that the oxidation product in this case was in fact the *o*-quinone product and not the *p*-quinone. With the nitrogen lone-pair delocalized through carbonyl resonance, oxygen lone-pairs are available to stabilize intermediate radical-cations and control the location of attack by water leading to quinone formation. For the desired *p*-quinone to form, water must attack at the C4 carbon between the alkyl group and the amide nitrogen. Evidently in this case, water attacks at C2 generating a carbonyl at that position leading exclusively to *o*-quinone formation.

Lack of *p*-quinone formation may be rationalized in the case of the model system **51**, yet the question of aza-quinone formation with lactam **1** for the synthesis of geldanamycin remains (Scheme 14). A stereoelectronic argument is difficult in this case due to the multiple low energy conformations that both GA and the synthetic intermediates adopt. A clue may be found in the Hsp90-bound conformation of GA as shown.[8] The amide nitrogen is found in a twisted *s*-cis conformation where the lone-pair on nitrogen is not fully conjugated to the carbonyl oxygen due to the constraints of the large ring. Instead of the usual planar dihedral angle of 0°, in this case the

SCHEME 14



SCHEME 15



out of plane twisting shows an angle of 12°. This conformation suggests that the amide nitrogen may electronically approximate the donation ability of an aniline type nitrogen with the potential for cation stabilization upon ring oxidation. The less electronegative nitrogen, compared to oxygen, may then be more able to donate its lone pair to the ring leading to aza-quinone formation selectively over either the *p*- or *o*-quinone products. In the case of the acyclic model **51**, the amide is flat and the N-lone pair is not available.

To overcome this electronic conformational bias, a strategy was devised to further delocalize the nitrogen lone-pair and favor formation of the desired quinone (Scheme 15). Following urethane formation with **45**, treatment with excess *tert*-butyloxycarbonyl anhydride (BOC_2O) in the presence of DMAP gave the *N*-BOC-

(36) The conditions found in ref 4b were used.
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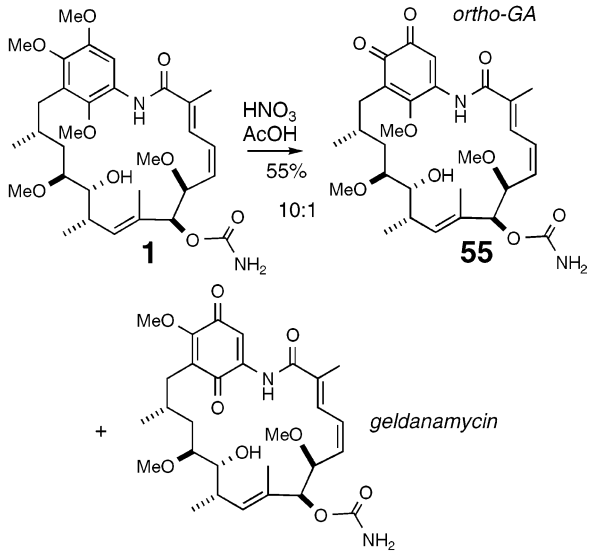
(40) X-ray data: orthorhombic space group *P*212121, $a = 7.359$ Å, $b = 8.645$ Å, $c = 21.12$ Å, independent data $R1 = 0.038$. Full details of the synthesis of **51** will be reported elsewhere.

SCHEME 16



protected lactam **53** in good yield.[41] The urethane was also BOC protected under these conditions. Treatment with CAN indeed gave a quinone type product **54** in low yield from **53**. No aza-quinone was observed. The quinone could not be definitively assigned as either para or ortho on the basis of the available data at this point. All conditions attempted in this case for BOC removal, dilute HCl,[42] HF, or the very mild $Mg(ClO_4)_2$,[43] gave only decomposition not geldanamycin.

Following a reference by Musgrave, use of nitric acid alone was attempted for the demethylative oxidation of **1** (Scheme 16).[44] A glacial acetic acid solution of **1** was treated for only 1 min with 70% nitric acid followed by quenching with sodium bicarbonate. Longer reaction times in this case led to extensive decomposition. The more polar, red-orange *o*-geldanamycin **55** (UV λ_{max} 266, 303, 431 nM) was the major product 10:1 over the less polar, yellow-orange desired *p*-quinone geldanamycin, which was shown to be identical in all respects (NMR, TLC, UV λ_{max} 260, 311, 422 nM) to an authentic sample. Multiple 10 mg runs were performed with reproducible results. The success of nitric acid over the other conditions may be attributed to protonation of the amide prior to oxidation, rendering the nitrogen lone pair less available for cation stabilization. As with the model substrate **51**, lactam **1** also prefers *o*-quinone formation. The route provided ample material for biologically evaluation of late-stage analogues.

TABLE 1. Cytotoxicity and Hsp90 Binding

compd	$SKBr_3$ IC_{50} (nM)
GA	40
o-GA-***55***	1272
tri-MeO-GA-**1**	>5000
aza-GA-**49**	838

Shown in Table 1 are cytotoxicity results for GA, *o*-quino-GA **55**, trimethylbenzene **1**, and the azaquinone **49**. The cell assays were performed with SKBr3 human cancer cells known to rely on Hsp90 dependent kinase signaling.[6] *o*-Quino-GA **55** showed comparable binding to GA, but was far less potent in the cell assay. The azaquinone **49** showed reasonable, but less potent, activity in the cell assay. The trimethylbenzene **1** was the least effective.

The total synthesis of geldanamycin has been accomplished for the first time. The linear route involved 41 steps from 1,2,4-trimethoxybenzene. Key reactions include an asymmetric dioxanone aldol reaction to set the anti stereochemistry at C11,12 and a norephedrine-based glycolate aldol step for the syn functionality at C6,7. Azaquinone was formed under the standard oxidation conditions, instead of the desired *p*-quinone product apparently due to ring-size induced amide nitrogen lone-pair donation. Nitric acid was used to form *o*-GA and geldanamycin without formation of the azaquinone product. New analogues of geldanamycin were tested for biological activity. These findings will lead to improved routes to geldanamycin including selective approaches to the *p*-quinone, development of a more convergent, nonlinear route, and analogues with improved Hsp90 binding.

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

JO034870L

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