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Synthesis and evaluation of 8,9-amido analogs of geldanamycin

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ABSTRACT

Amido analogs of geldanamycin, an ansamycin anticancer agent, were designed, synthesized, and assayed with SKBR3 cells, in which stability of HER2 receptor tyrosine kinase is dependent on the chaperone Hsp90. An amide was employed as a trisubstituted alkene isostere at the C8,9 position, which provided for a simplified, convergent synthesis through two major fragments, an aniline-amine left-hand portion and a dicarboxylic acid right-hand piece.

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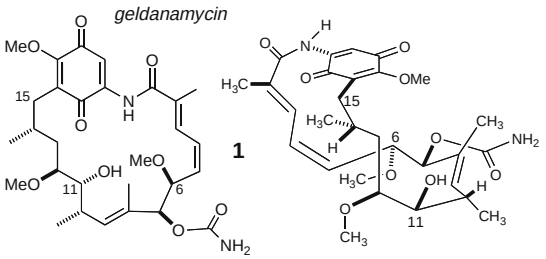
We recently reported the total synthesis of the ansamycin antitumor antibiotic geldanamycin 1 (GA).[1] Isolated (*Streptomyces hygroscopius geldanus)* in 1970 by workers at Upjohn with the structure determined by Rinehart shortly thereafter,[2] it continues to attract considerable attention as a starting point for anticancer therapeutic design.[3] Ansamycins, including the related herbamycin A and macbecin I, possess various biological activities, including potential as antibacterial and anticancer agents.[4] Various semi-synthetic analogs have been made and tested. Among these, the clinical agent 17-allylamino-GA is the most prominent.[5] GA demonstrated a unique profile of action with the NCI 60 cell-line panel with an average ED_{50} of 180 nM.[6] Neckers, via an affinity protocol demonstrated that geldanamycin binds to the chaperone heat-shock protein 90 (Hsp90).[7] Subsequently GA was shown to selectively reduce the stability of several oncogenic tyrosine kinases, including v-Src, Bcr/Abl, and HER2, by interfering with their association with Hsp90. X-ray structures for the Hsp90-GA complex show that GA binds to an ATP site in a C-clamp conformation that is distinct from its free, solution conformation (Scheme 1).[8] In an effort to access this bound conformer and to simplify the synthesis, amido isostere analogs of GA were envisioned. We now report the synthesis and SKBR3 cell assay activity of two 8,9-amido variations **2** and **3** assembled in a convergent manner from two key pieces, an aniline amine and a dicarboxylic acid produced using asymmetric glycolate aldol methodology.[9]

An amido moiety was selected as a trisubstituted alkene mimic to provide a rigid *s*-trans conformer and a critical disconnection point for the synthesis (Scheme 2). Synthesis of this alkene region has proven to be problematic being located between five stereocenters with labile functionality.[1,10] Amido analogs **2** and **3** were designed with the amide located at the 8,9-position with the carbonyl oxygen to approximate the C8-methyl group of GA and the N–Me or N–H groups pointing down at the 9-position in the semi-rigid *s*-trans conformer. The C10 methyl is included in analog **2** and removed in **3** allowing for the use of a primary amine precursor. The more flexible analog **3** was envisioned to more readily access the bound conformer. The C14 methyl group was also inverted to the *S*-stereochemistry. The natural *R*-C14 methyl appears to adopt a pseudo axial position directed toward the interior of the bound conformer located under the quinone. The design was guided by B3LYP/6-31G* calculations showing that these initial analogs were more able to adopt the bound conformation compared to GA.[11] The bound conformer of **2** was +7.4 and **3** was +6.3 kcal/mol higher in energy compared to GA which was



Scheme 1.

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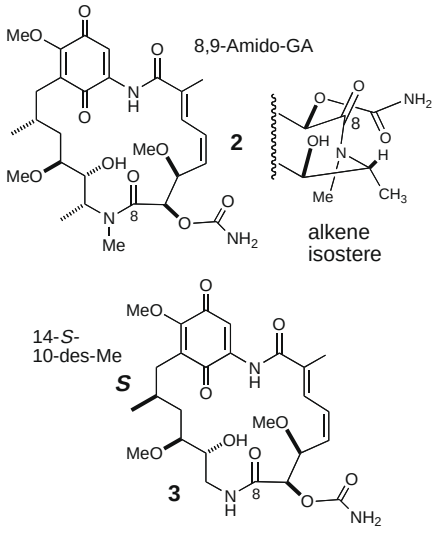


Scheme 2.

+14 kcal/mol higher than its unbound form. An amide isostere also provides a more polar alternative to GA, which suffers from low water solubility.[12]

The synthesis follows a convergent approach that utilizes protected aniline amines **4a** and **4b** coupled with allyl ester carboxylic acid **5** (Scheme 3). These two key intermediates possess vicinal methoxyhydroxy functionality at C6,7 and C11,12, which are addressed with efficient asymmetric glycolate aldol methods.[9]

The synthesis of the left-hand pieces **4a** and **4b** began with *R*-**6a** and *S*-**6b** (Fig. 1) individually produced using asymmetric Evans alkylations in 10 steps from methoxydihydroquinone as previously reported in the synthesis of GA.[1] Anti-glycolate aldol reactions were performed using *S,S*-**7** and our general method with dicyclohexylboron triflate and triethylamine to give *S,S*-dioxanones **8a** and **8b**.[9b]

Methyl ether formation and transesterification then gave esters **9a** and **9b** in high overall yield (Fig. 1). Compound **9a** was then converted to the aldehyde **10** in three steps. Methyl ketone **11** was then formed using trimethylaluminum addition followed by Dess–Martin periodinane oxidation.[13] Weinreb amides were also formed; however, they did not produce **11** under methyl Grignard or methyllithium conditions. At this point it became necessary to reduce the aryl nitro and form the Alloc-aniline **12** (allyloxycarbonyl). This hydrogenation step required the use of isopropyl alcohol. The more common solvents for hydrogenation, methanol or ethanol with **11**, gave rise to *N*-methyl and *N*-ethyl products presumably formed through imines generated from in situ-produced formaldehyde and acetaldehyde via Pd-mediated oxidation. With isopropyl alcohol, the slower rate of acetone and imine formation allows for efficient primary aniline generation. Methylimine formation and selective reduction were performed with the methyl ketone **12** using a modification of reported conditions with titanium tetra-isopropoxide and methylamine followed by addition of $NaBH_4$ at −78 °C.[14] In accord with a chelate model, the *R*-stereocenter was selectively produced (20:1) in good yield for amine **4a**.

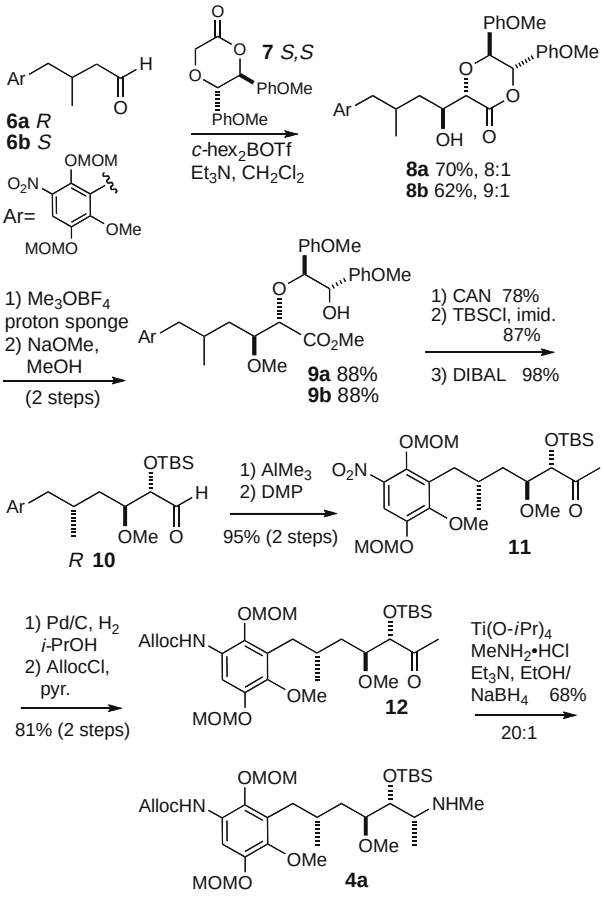
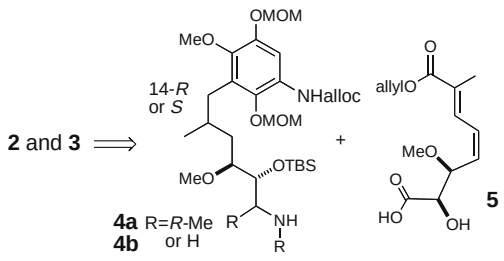


Figure 1. Left-hand piece **4a** formation.

The synthesis of **4b** (Fig. 2) began in similar fashion with CAN (cerric ammonium nitrate) removal of the benzyl ether of **9b**,[9b] protection with *t*-butyldimethylsilyl chloride, and reduction with Pd/C to the aniline **13**. The alloc group was then installed and reduction to the primary alcohol gave **14**. The azide was attached



Scheme 3.

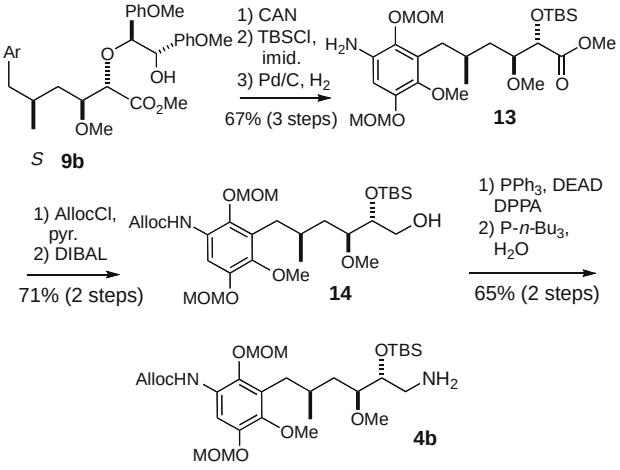


Figure 2. Synthesis of the primary amine **4b**.

using Mitsunobu DEAD (diethyl azodicarboxylate) conditions with DPPA (diphenylphosphoryl azide).[15] Subsequent treatment with tri-*n*-butylphosphine and water generated the free amine **4b** in good overall yield.

The right-hand fragment **5** was generated from PMB (*p*-methoxybenzyl) aldehyde **15** (Fig. 3). The Masamune norephedrine glycolate **16**, developed previously for *syn*-diol formation,[9c] was employed with dicyclohexylboron triflate to generate product **17** in high yield and excellent selectivity. Protection with TESCl (triethylsilyl chloride) and reduction with DIBAL at low temperature gave aldehyde **18**. A Touchard-modified Ando phosphonate **19** was employed using NaI and TMG (tetramethyl guanidine) as base to give the *Z*-ester **20** in near quantitative yield and selectivity.[16] The corresponding hexafluorophosphonate gave a lower yield with reduced selectivity in this case. DIBAL (diisobutylaluminum hydride) and DMP oxidation gave *Z*-enal **21**. The allylester phosphonate was employed to produce the *E,Z*-diene ester in high yield with complete selectivity. The PMB group was removed with DDQ (dicyanodichloroquinone) followed by oxidation to the aldehyde and finally to the carboxylic acid with $NaClO_2$ with sodium biphosphate and 2-methyl-2-butene. This final step also incurred TES ether removal. Oxidation from the primary alcohol directly to the acid in this case was problematic using a variety of standard reagents.

At this point various vinylogous phosphonates[17] were explored in an effort to directly install the *E,Z*-diene with aldehyde **18** (Fig. 4). The most promising result was obtained using allylester bis-trifluoroethyl phosphonate **24**. Optimized conditions gave diene **23** in only modest yield and 4:1 selectivity. The analogous Ando phosphonate, with *o*-*t*-butylphenyls in place of the trifluoroethyl groups,[16] was also made and investigated. Unfortunately, it gave a poor 1:1 mixture of products **23**. It was thus determined to use the selective, step-wise approach to produce **5** (Fig. 3).

The amines **4a** and **4b** were coupled with acid **5** using HATU (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetra methyl uronium hexafluorophosphate)[18] and Hünigs base in methylene chloride at 0 °C to give amides **25a** and **25b** in efficient yields (Fig. 5). Other reagents, including PyBrop, BOP-Cl, and DEPC (diethyl phosphocyanidate) proved to be ineffective. The alloc group was removed

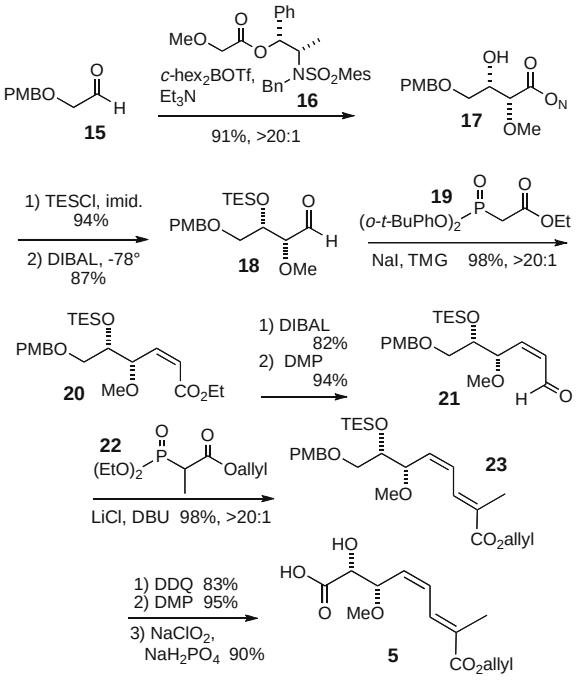


Figure 3. Synthesis of *E,Z*-diene carboxylic acid **5**.

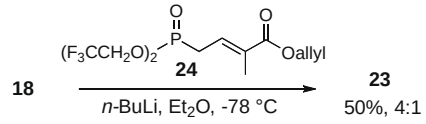


Figure 4. Direct approach to *E,Z*-diene **23** with allylphosphonate **24**.

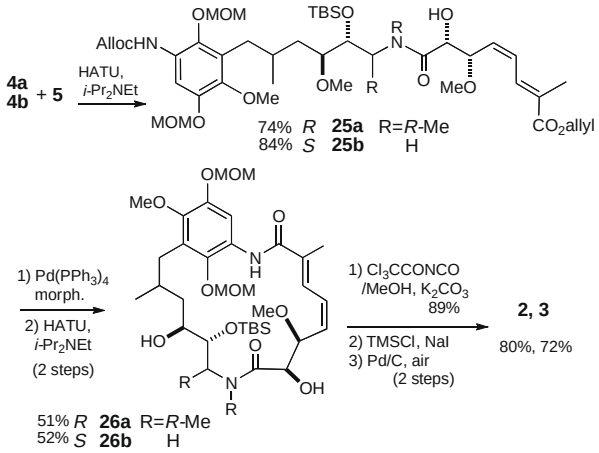


Figure 5. Coupling and completion of the C8,9-amide GA routes.

from the aniline nitrogen and the allyl ester was converted to the carboxylic acid in the same step with palladium tetrakistriphenylphosphine (40 mol %) and excess morpholine in THF. The intermediate was isolated and treated again with HATU, Hünigs base, and DMAP (4-*N,N*-dimethylaminopyridine) in methylene chloride under dilute conditions (0.001 M). The lactams **26a** and **26b** were isolated in good yields, 51% and 52%. Treatment with trichloroacetyl isocyanate and methanol with potassium carbonate installed the C7-carbamate.[1] And finally, TMSI from TMSCl and sodium iodide was used to remove the two MOM ethers followed by Pd/C exposed to air,[19] which gave the desired *p*-quinone C8,9-amide GA analogs **2** and **3** in good yields. The 1,4-di-MOM-protected substrate is a significant improvement over the original route to GA where a tri-methoxybenzene precursor gave primarily an *ortho*-quinone product upon oxidation.[1] The routes to **2** and **3** involve 38 total steps with the longest linear sequence of 26 and 25 steps, respectively. This is an improvement in efficiency of 16 steps compared to the synthetic route to GA **1** which required 41 linear steps.

The impact of **2** and **3** on HER2 stability in SKBR3 cells was examined and compared to GA **1**.[20] This assay has been used extensively to query the activity of new Hsp90 inhibitors. Unfortunately, the results in this case indicated that **2** and **3** are significantly less potent in promoting HER2 degradation (>20 μM) compared to GA (ED_{50} 5 nM). NOE experiments performed to access solution conformations proved inconclusive (<1%). While it remains difficult to determine which individual factors contributed to lowered activity in this case, a convergent approach has been developed to access more polar analogs of this challenging anticancer agent and this more efficient route may yet play a role in the development of more suitable variants with optimal activity.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures, characterization, and NMR data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.09.091.

References and notes

1. (a) Andrus, M. B.; Meredith, E. L.; Simmons, B. L.; Soma Sekhar, B. B. V.; Hicken, E. J. *Org. Lett.* **2002**, *4*, 3549; (b) Andrus, M. B.; Meredith, E. L.; Hicken, E. J.; Simmons, B. L.; Glancey, R. R.; Ma, W. *J. Org. Chem.* **2003**, *68*, 8162.
2. (a) De Boer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* **1970**, *23*, 442; (b) Rinehart, K. L.; Sasaki, K.; Slomp, G.; Grostic, M. F.; Olson, E. C. *J. Am. Chem. Soc.* **1970**, *92*, 7591.
3. (a) Sharp, S.; Workman, P. *Adv. Cancer Res.* **2006**, *95*, 323–348; (b) Rastelli, G.; Tian, Z. Q.; Wang, Z.; Myles, D.; Liu, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5016; (c) Hadden, M. K.; Blagg, B. S. *J. Org. Chem.* **2009**, *74*, 4697; (d) Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N. *J. Am. Chem. Soc.* **2005**, *127*, 6999.
4. Floss, H. G. *J. Nat. Prod.* **2006**, *69*, 158.
5. Drysdale, M. J.; Brough, P. A. *Curr. Top. Med. Chem.* **2008**, *8*, 859.
6. Whitesell, L.; Shifrin, S. D.; Schwab, G.; Neckers, L. M. *Cancer. Res.* **1992**, *52*, 1721.
7. Whitesell, L.; Mimnaugh, E. G.; De Costa, B.; Myers, C. E.; Neckers, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324–8328.
8. (a) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239; (b) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65.
9. (a) Andrus, M. B.; Soma Sekhar, B. V.; Meredith, E. L.; Dalley, N. K. *Org. Lett.* **2000**, *2*, 3035–3037; (b) Andrus, M. B.; Mendenhall, K. G.; Meredith, E. L.; Soma Sekhar, B. B. V. *Tetrahedron Lett.* **2002**, *43*, 1789–1792; Andrus, M. B.; Soma Sekhar, B. B. V.; Turner, T. M.; Meredith, E. L. *Tetrahedron Lett.* **2001**, *42*, 7197–7201; (c) Khartulyari, A. S.; Kapur, M.; Maier, M. E. *Org. Lett.* **2006**, *8*, 5833; (d) Zhou, X. –T.; Lu, L.; Furkert, D. P.; Wells, C. E.; Carter, R. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7622.
10. For recent routes to herbimycin A: (a) Carter, K. D.; Panek, J. S. *Org. Lett.* **2004**, *6*, 55; (b) Canova, S.; Bellosta, V.; Bigot, A.; Mailliet, P.; Mignani, S.; Cossy, J. *Org. Lett.* **2007**, *9*, 145; Macbecin I: (c) Panek, J. S.; Xu, F. *J. Am. Chem. Soc.* **1995**, *117*, 10587; (d) Panek, J. S.; Xu, F.; Rondon, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 4413; (e) Belardi, J. K.; Micalizio, G. C. *Angew. Chem., Int. Ed.* **2008**, *47*, 4005; Geldanamycin: (f) Qin, H. L.; Panek, J. S. *Org. Lett.* **2008**, *10*, 2477.
11. Bound structures were constrained to the C-clamp conformation approximating binding to the ATP-site. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. A.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N. *GAUSSIAN 03, Revision B.03*, Gaussian: Pittsburg, PA.
12. Supko, J. G.; Hickman, R. L.; Grever, M. R.; Malspeis, L. *Cancer Chemother. Pharmacol.* **1995**, *36*, 305–315.
13. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4156.
14. (a) Kumpaty, H. J.; Bhattacharyya, S. *Synthesis* **2005**, 2205; (b) Miriyala, B.; Bhattacharyya, S.; Williamson, J. S. *Tetrahedron* **2004**, *60*, 1463.
15. Wilk, B. *Synth. Commun.* **1993**, *23*, 2481.
16. (a) Touchard, F. P. *Eur. J. Org. Chem.* **2005**, 1790; (b) Ando, K. *J. Org. Chem.* **1999**, *64*, 8406.
17. (a) Nakata, M.; Osumi, T.; Ueno, A.; Kimura, T.; Tamai, T.; Tatsuda, K. *Tetrahedron Lett.* **1991**, *32*, 6015; (b) Chemler, S. R.; Cofey, D. S.; Roush, W. R. *Tetrahedron Lett.* **1999**, *40*, 1269.
18. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
19. Andrus, M. B.; Hicken, E. J.; Meredith, E. L.; Simmons, B. L.; Cannon, J. F. *Org. Lett.* **2003**, *5*, 3859.
20. Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273–279.