Natural Product Synthesis

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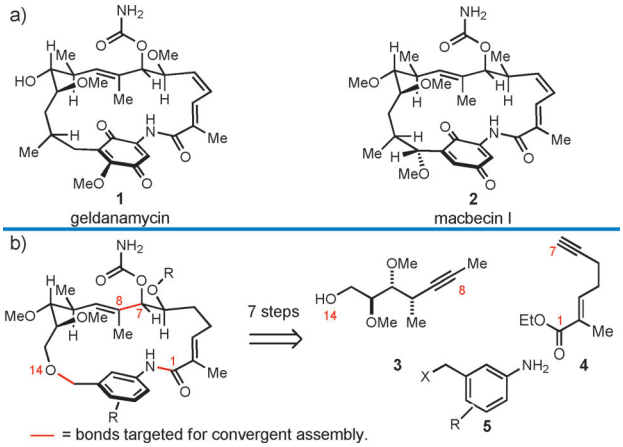
Synthesis of Benzoquinone Ansamycin-Inspired Macrocyclic Lactams from Shikimic Acid

Valer Jeso, Sarah Iqbal, Pamela Hernandez, Michael D. Cameron, HaJeung Park, Philip V. LoGrasso, and Glenn C. Micalizio**

The benzoquinone ansamycins, exemplified by geldanamycin and macbecin (Scheme 1 a), are notable examples of natural products which continue to serve as inspiration for the development of clinically relevant anticancer agents.[1] While initially reported as a natural product with potent antibiotic properties, geldanamycin was later described as the first small-molecule inhibitor of Hsp90 and has grown as a pharmacological tool, the use of which has supported claims of the essential role that stress proteins play in oncogenic transformation.[2,3] Benzoquinone ansamycins are polyketide-derived natural products possessing a molecular framework which continues to challenge modern organic chemistry. The first total synthesis of a natural product in this class was reported nearly twenty-five years ago and delivered macbecin by a chemical pathway which required over forty sequential transformations.[4a,b] Since then, scores of reports have appeared and they describe conceptually unique entries to targets within this class.[4c–o] While recent advances have led to total syntheses of benzoquinone and benzenoid ansamycin natural products in less than twenty linear steps,[4n,o] chemical synthesis has still not risen as an enabling technology to drive the discovery or development of benzoquinone-ansamycin-inspired agents. In fact, state-of-the-art pursuits targeting the development of natural product-like Hsp90 inhibitors embrace semisynthesis (natural product derivatization) and engineered biosynthesis,[5] both of which come with substantial molecular limitations. Perhaps stemming from such limitations, much pharmaceutical effort has turned away from the benzoquinone ansamycin skeleton in favor of synthetically tractable small-molecule Hsp90 inhibitors (i.e., resorcinol and purine derivatives).[6] Herein, we describe a scientific pursuit which has resulted in the development of a robust and stereoselective pathway to natural product-inspired agents from shikimic acid, a route which is exquisitely selective, flexible, and convergent, thus delivering complex macrocyclic lactams in just seven steps from simple coupling partners (**3**–**5**; Scheme 1 b). In addition to describing the chemistry capable of delivering such macrocycles, our efforts have led to the discovery of a natural product-inspired skeleton which is uniquely isoform selective (in comparison to natural products and known analogues), thus favoring inhibition of cytosolic and nuclear Hsp90 over the ER-associated Grp94, and possesses quite potent anticancer properties (perhaps by a novel mechanism of action).



Scheme 1. Introduction. a) Representative benzoquinone ansamycins. b) Natural product-inspired macrocyclic lactams from convergent assembly of fragments **3**, **4**, and **5**.

Our efforts began with an understanding of structure–activity relationships associated with benzoquinone ansamcyins established through semisynthesis, and an inspection of the geldanamcyin/Hsp90 X-ray crystal structure (Figure 1).[7] First, it is well established that the quinone of geldanamycin does not play an important role in binding to Hsp90. In fact, it has been proposed that its presence is a substantial liability which confers hepatic toxicity and serves as a locus of enzyme-mediated redox cycling which defines a mechanism for acquired resistance.[6a,8] As such, our early designs aimed to establish a chemical pathway capable of delivering agents, which have a diverse range of systems, in place of the natural product's quinone. Further, in an effort to define a highly convergent approach to the assembly of natural product-inspired macrolactams, we searched for a site in the carbon backbone which would tolerate an atomic mutation from a carbon atom to a suitable heteroatom.[9] In this way, convergent assembly could ensue by facile C–X (i.e., X = O, N, or S) rather than C–C bond formation. We suspected that C14 would represent an ideal candidate, as the stereodefined methine unit of the natural product does not appear to have a substantial impact on conformation or offer a significant interaction with Hsp90 (the Me-group stemming from C14 is

[*] V. Jeso, Prof. G. C. Micalizio
Department of Chemistry, The Scripps Research Institute
130 Scripps Way #3A2, Jupiter, FL 33458 (USA)
E-mail: micalizio@scripps.edu
S. Iqbal, P. Hernandez, M. D. Cameron, H. Park, Prof. P. V. LoGrasso
Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL 33458 (USA)
E-mail: lograsso@scripps.edu

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positioned inside the macrocycle in its Hsp90-relevant conformation; Figure 1 B).

Our planning to execute the retrosynthetic strategy previously depicted in Scheme 1 b began with securing

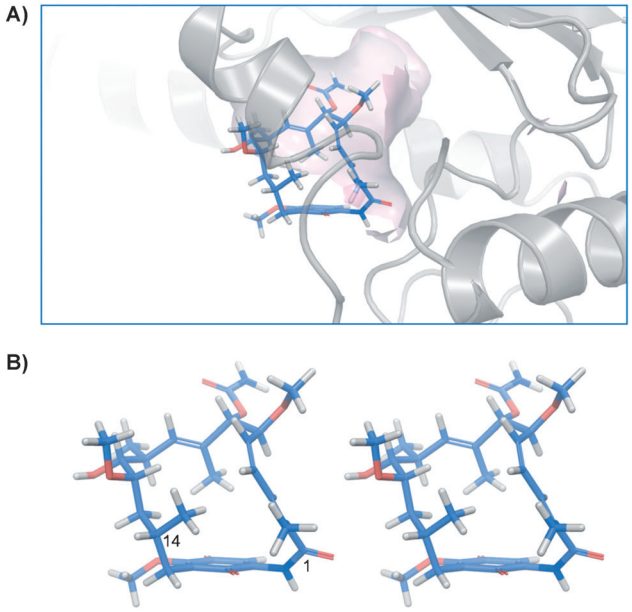


Figure 1. A) The geldanamcyin/Hsp90 complex (PDB ID 1YET). B) Stereoview of the Hsp90-relevant conformation of geldanamycin.

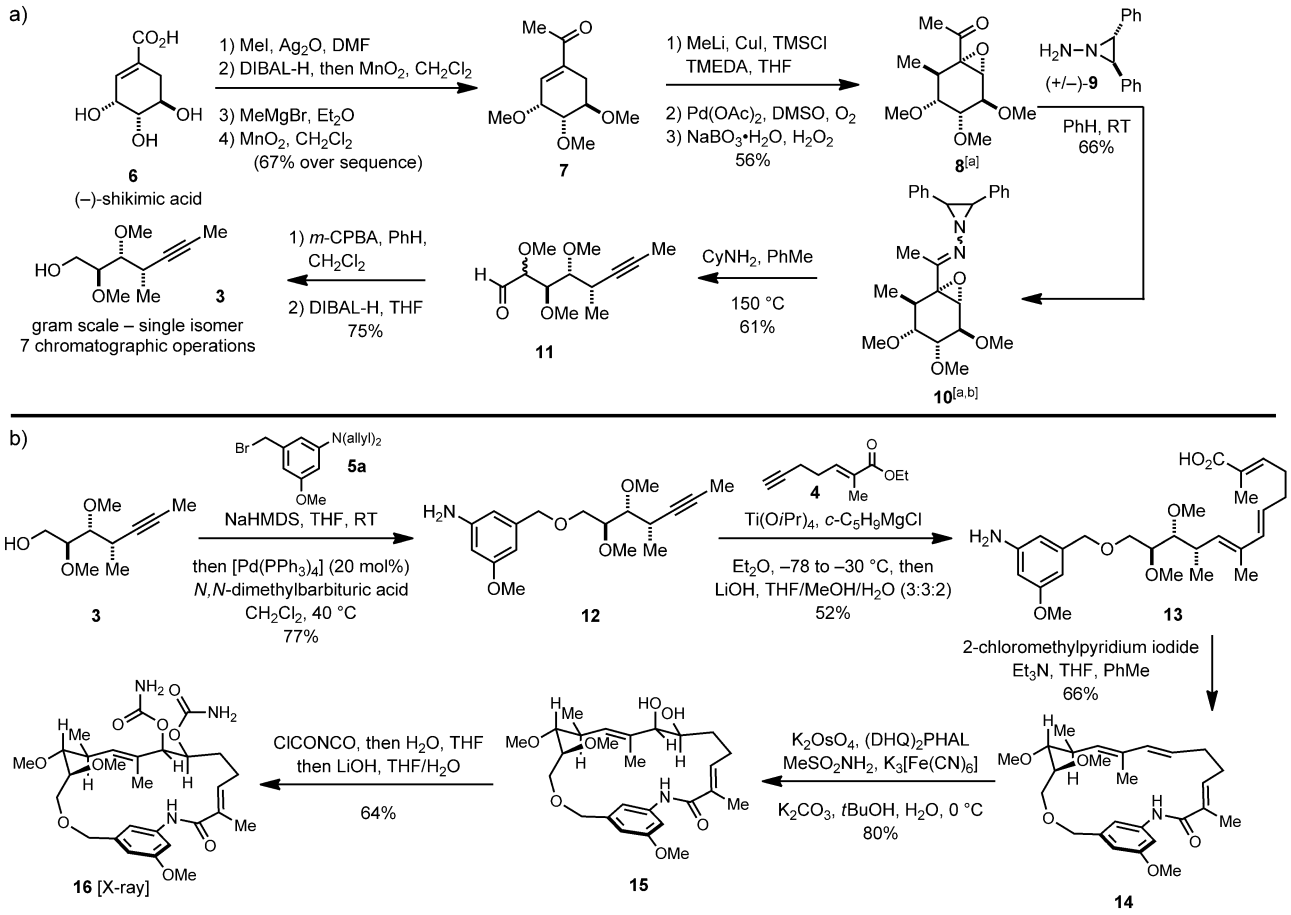
a robust entry to the stereodefined internal alkyne-containing primary alcohol **3**. While many methods in acyclic stereocontrol can be considered to address this target, our previous experience in pursuing a related subunit in our total synthesis of macbecin greatly impacted our planning.[4n,9] Asymmetric enolate alkylation and stereoselective propargylation chemistry was successful in our early investigations, but use of these processes resulted in a laborious sequence which required great attention to be focused on the removal of minor isomers which arose in each of these transformations. In an effort to establish a concise and highly stereoselective synthesis of this region of the macrocyclic skeleton, we focused our attention on embracing shikimic acid (**6**) as a stereochemically rich starting material which could be converted into **3** in a straightforward manner (Scheme 2 a). Exhaustive methylation (MeI, Ag_2O, DMF) was followed by conversion into the α,β-unsaturated methyl ketone **7**.[10] Stereoselective 1,4-addition then proceeded by exposure to the combination of MeLi, CuI, and TMSCl in TMEDA/THF, thus resulting in the formation of the TMS enol ether with outstanding levels of stereoselectivity (d.r. ≥ 20:1). Saegusa oxidation[11] of this intermediate [$Pd(OAc)_2$, DMSO, O_2] proceeded in a regioselective fashion and delivered the less substituted enone product (no evidence could be found for the regioisomeric product). Next, attempts to convert the enone product into an epoxy ketone intermediate were initially problematic, as treatment with NaOH and H_2O_2 led to a complex mixture of products. After extensive experimentation, it was found that treatment with $NaBO_3{\cdot}H_2O$ and H_2O_2 in H_2O led to effective generation of the desired epoxide **8** as a single stereoisomer.[12]

With the stereochemically rich carbocycle **8** in hand, effort was directed to the fragmentation of this species to deliver the desired acyclic alkyne-containing coupling partner. Extensive experimentation in the search of suitable reaction conditions to promote an Eschenmoser–Tanabe fragmentation[13] led to securing a pathway to the alkyne **11** through the intermediacy of an activated hydrazone (**10**). In short, exposure of ketone **8** to the aziridinyl hydrazine (±)-**9** resulted in the formation of **10** which on treatment with $CyNH_2$ in toluene at 150 °C underwent the desired fragmentation to produce **11** as a mixture of stereoisomers. Baeyer–Villiger oxidation[14] (*m*-CPBA, CH_2Cl_2, PhH) then furnished an intermediate formate ester which was reduced to the corresponding primary alcohol **3** by the action of DIBAL-H. Notably, this pathway to the chiral coupling partner **3** proceeded through a sequence which required only seven chromatographic operations and delivered the desired product as a single stereoisomer.

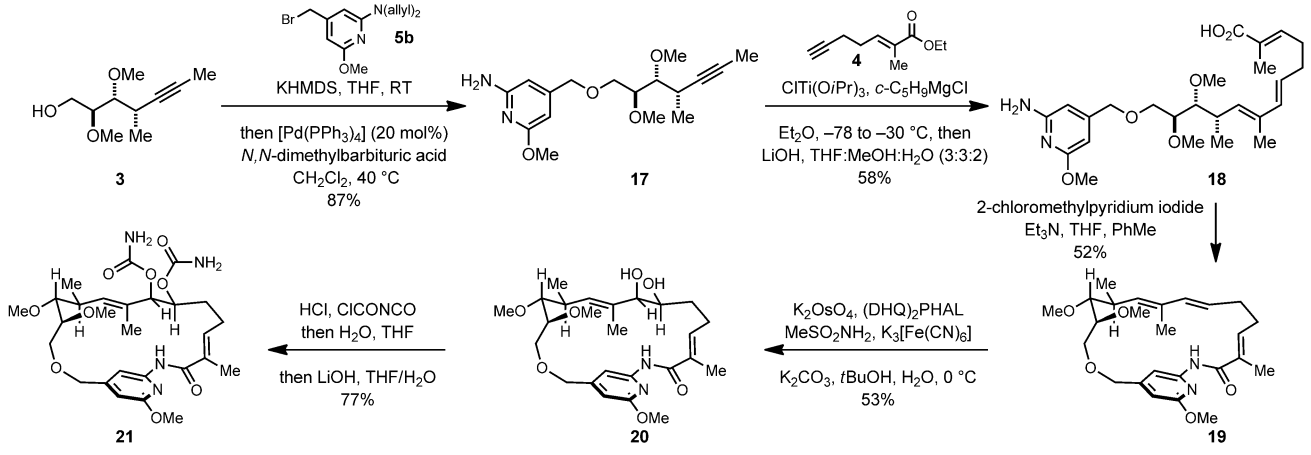
As illustrated in Scheme 2 b, etherification with the benzylic bromide **5 a** and subsequent palladium-catalyzed deprotection of the aniline furnished the benzyl ether **12** in 77 % yield. Coupling with the enyne **4** was then accomplished in a reductive manner by a titanium-mediated process[15] to deliver a stereodefined triene which was further advanced to the seco acid **13** in 52 % overall yield (regioselectivity for the reductive alkyne–alkyne coupling was 8:1). Macrocyclization promoted by Mukaiyama's reagent then delivered the macrocyclic triene **14** in 66 % yield.[16] Site- and stereoselective dihydroxylation using the Sharpless system produced the complex diol **15** in 80 % yield as a single isomer.[17] Finally, carbamate formation with ClCONCO and subsequent hydrolysis furnished the fully functionalized natural product-inspired macrolactam **16** in 64 % yield, and the structure was confirmed by X-ray crystallography.[18]

To demonstrate the flexibility of this convergent synthesis pathway, we explored the potential of the process to deliver macrocyclic analogues which possess a heterocycle in place of the aniline resident in **16**. These efforts are summarized in Scheme 3 and support the conclusion that the key coupling reactions for this convergent synthesis are robust and tolerant of varied functionality in the aromatic nucleus. Further, in pursuits to generate macrocyclic lactams with a tertiary amine located at position 14, we found that with only simple modifications of this synthesis pathway that such complex natural product-inspired agents can be readily accessed (Scheme 4).

Notably, these pursuits have led to the establishment of a robust and straightforward convergent synthesis pathway to natural product-inspired macrocyclic lactams and have demonstrated the great functional-group compatibility and selectivity associated with titanium-mediated alkyne–alkyne reductive cross-coupling. For example, this coupling reaction has been successful with substrates bearing free anilines and aminopyridines, as well as with terminal alkynes which possess an α,β-unsaturated ester. The syntheses proceeds in approximately seven steps from the relatively simple coupling partners, the most sophisticated of which is prepared in



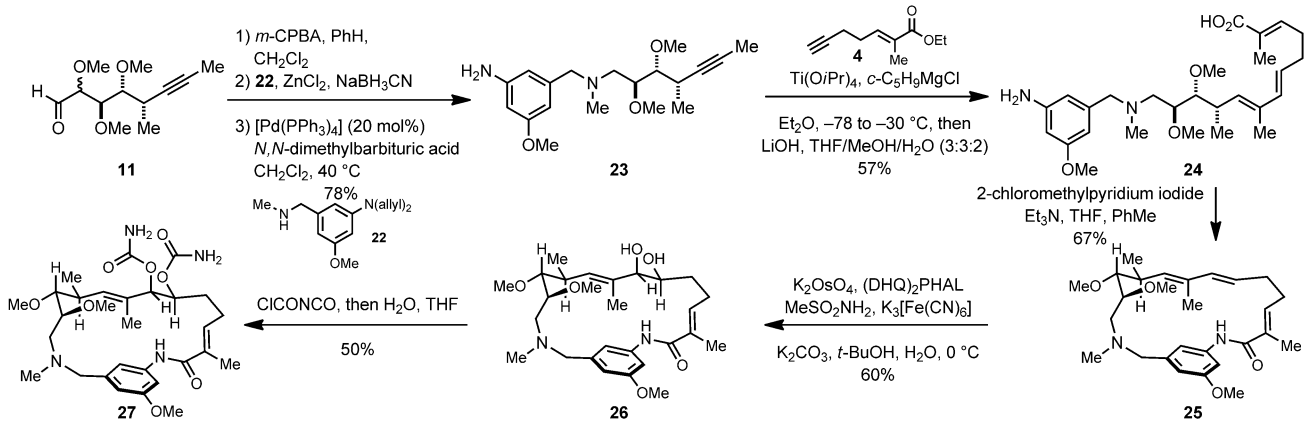
Scheme 2. Synthesis of natural product-inspired macrocyclic lactams. a) From shikimic acid to coupling partner **3**. b) Convergent synthesis of macrocyclic lactams. [a] Stereochemistry of the epoxide is assumed [b] Product contains a mixture of isomeric aziridines derived from (±)-**9**. DIBAL-H = , HMDS = hexamethyldisilazide, *m*CPBA = *meta*-chloroperbenzoic acid, THF = tetrahydrofuran, TMEDA = tetramethylethylenediamine, TMS = trimethylsilyl.



Scheme 3. Synthesis of a pyridine-containing natural product-inspired macrocyclic lactam.

a straightforward and highly stereoselective sequence from shikimic acid.

While our pursuits have been focused on defining chemistry suitable to populate "chemical space" defined by the benzoquinone ansamycins, we have begun to explore the anticancer properties of the natural product-inspired agents derived from these pursuits. Preliminary experiments have demonstrated that the aminopyridine- and tertiary amine-containing macrocycles (**21** and **27**) are ineffective at displacing fluorescently labeled geldanamycin from Hsp90 (at



Scheme 4. Synthesis of a natural product-inspired macrocyclic lactam with a main chain tertiary amine at position 14 of the macrocycle.

concentrations up to 30 μM),[18] and that **16** possesses interesting properties. While geldanamycin has a *pan*-inhibitory profile versus Hsp90 and Grp94,[18] we find that **16** has rather profound isoform selectivity favoring association with Hsp90α and Hsp90β over Grp94 by about two orders of magnitude (1 vs. > 100 μM). While the affinity of **16** to Hsp90 is about one order of magnitude lower than that observed for geldanamycin (1 μM vs 100 nM),[18] these studies mark a compelling starting point for the development of isoform-selective Hsp90 inhibitors.[19] Furthermore, in vitro experiments have revealed an intriguing natural product-like anticancer profile associated with **16**. For example, the IC_{50} values for **16** versus MCF7 and HeLa cells were nearly identical to those of geldanamycin (**16**: 85 and 400 nM; geldanamycin: 93 and 617 nM). The origin of this similar toxicity profile may speak to an as yet unidentified and potentially interesting target of **16**, or a unique effect that **16** has on the action of Hsp90 in vitro. These questions remain unanswered and will be the topic of future exploration.

Finally, we have determined the structure of **16** complexed with Hsp90 and find that it adopts a conformation much like that seen in the geldanamcycin/Hsp90 structure (Figure 2).[18] We look forward to the use of this structural data and the chemical synthesis pathway described herein to drive a program aimed at the design and synthesis of natural product-inspired anticancer agents.

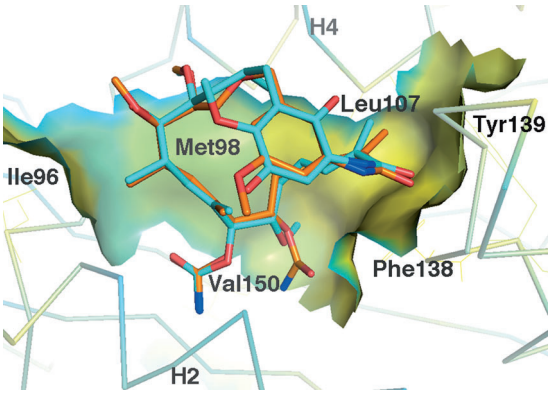


Figure 2. Crystal structure of **16** bound to Hsp90 (PDB ID 4JQL). An overlay of **16** (orange) and geldanamycin (cyan) shown with surface representation of hydropobic pocket in Hsp90.

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