



Design and synthesis of 3',5'-ansa-adenosines as potential Hsp90 inhibitors

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ABSTRACT

3',5'-Ansa-adenosine derivatives, rationally designed as an Hsp90 inhibitor by extracting and fusing a natural product, geldanamycin, and a natural substrate, ATP, were efficiently synthesized by the ring-closing metathesis assisted by the 2,4-dimethoxybenzyl group. This simpler scaffold design provides a practical synthesis of a set of analogs and demonstrates synthetic innovation.

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The heat shock protein 90 (Hsp90) family is a group of molecular chaperones that play a key role in the folding of polypeptide, called as client proteins.¹ Many of the Hsp90 client proteins are oncogenic and are also considered hallmarks of the disease.² Inhibition of Hsp90 leads to degradation of these various client proteins by a proteasome. The simultaneous combinatorial depletion of many cancer-causing client proteins and the modulation of all of the hallmarks of cancer are the major advantages of Hsp90 inhibitors. Geldanamycin (GDM, Fig. 1, **1**)³ is known to be an inhibitor of Hsp90 and exhibits anti-proliferation activity against a range of tumor cell lines.⁴ The 17-allylamino derivative (17-AAG, **2**) and the 17-dimethylaminoethylamino derivative (17-DMAG, **3**) of GDM are currently in phase II clinical studies for anti-cancer chemotherapy.⁵ However, it has been revealed that, as a potential drug, GDM has certain drawbacks such as solubility and toxicity. Chemical modifications of GDM are limited, raising the problem of providing a range of analogs by total synthesis.⁶ GDM is a competitive inhibitor with ATP, which is a natural substrate of the Hsp90 N-terminal domain.^{7,8} Interaction of GDM in the N-terminal ATP-binding pocket of Hsp90 has been revealed by X-ray crystal structure analysis.^{9,7c,10} The key features of the interaction upon binding may be described as follows: (a) the bound GDM adopts an overall folded conformation with a *cis*-amide bond, (b) the carbamate moiety at the 7-position mimics the adenine moiety with the key hydrogen bonding to the pocket, and (c) the benzoquinone moiety is found at the top of the binding pocket, where the β - and γ -phosphate groups of the ATP lie. As opposed to enzymes, such as protein kinases which utilize ATP as a substrate, the N-terminal ATP-binding pocket of Hsp90 adopts a rather unusual Bergerat fold.¹¹ When inside the pocket, the ATP adopts a U-shaped bend at the phosphate moiety and the 3'-*endo*-conformation at the ribofuranose moiety. The superimposition of the bound ATP and the bound GDM in the N-terminal ATP-binding pocket of Hsp90 provides additional information, where the 14- or 15-position of

GDM overlaps with the 3'-position of the ATP. Considering these structural features of ATP and GDM, we designed 3',5'-ansa-adenosine derivatives containing the benzoquinone moiety (Fig. 2). Although chimeric inhibitors of Hsp90 exemplified by two natural products, GDM and radicicol, have been reported,¹² there have been no Hsp90 inhibitors designed by extracting and fusing a natural product and a natural substrate. The aim of this study was to establish a synthetic route to this class of molecules, for example, **4**.

Scheme 1 describes the preparation of the phosphonate **9** bearing the *N*-aryl carbamoyl substituent, which was used in the Horner–Wadsworth–Emmons (HWE) olefination¹³ with the adenosine 5'-aldehyde derivative. 2-Methoxy-1,4-bis-methoxymethoxyphenyl-3-cuprate,¹⁴ which was prepared by an *ortho*-lithiation of **5** followed by metal exchange, was reacted with allyl bromide to give **6** in 66% yield. According to the procedure previously reported,¹⁴ a nitro group was introduced at the 5-position of **6** under mild conditions using ammonium nitrate and trifluoroacetic anhydride in THF at $-20\text{ }^{\circ}\text{C}$ to give **7** in 41% yield. The nitro group in **7** was reduced by zinc to afford the aniline **8** in 85% yield. The resulting amine **8** was acylated with diethyl phosphonoacetic acid and EDCI in the presence of DMAP to give the *N*-aryl phosphonoacetamide **9** in 95% yield.

The convergent assembly of the key *N*-aryl unsaturated compound **15** from **9** and the labile adenosine 5'-aldehyde derivative **14** is summarized in **Scheme 2**. The previous method for the preparation of 3'-*O*-allyl-adenosine **11** required a multi-step procedure, including a protection-deprotection sequence and chromatographic separation,¹⁵ and thus was not suitable for a large-scale synthesis. We prepared **11** simply by allylation of the 2',3'-*O*-stannyleneadenosine¹⁶ followed by crystallization. Although the yield was not high, this method allowed us to operate on a 0.5 mol scale to obtain pure **11** without any chromatography. 3'-*O*-Allyl-adenosine **11** was sequentially protected with TBS and Tr groups to give the suitably protected **12**. Selective removal of the TBS-protecting group at the 5'-hydroxy group was conducted by TBAF at low temperature to give the corresponding

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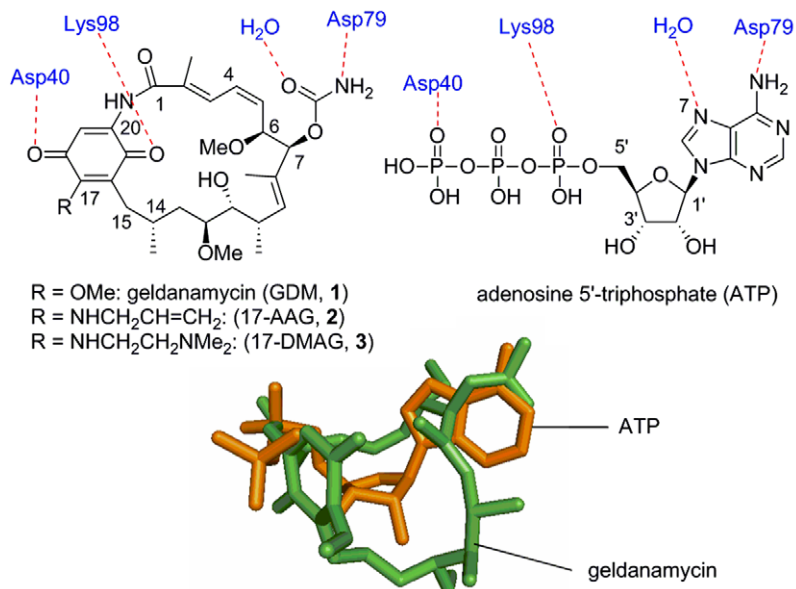


Figure 1. Structural comparison of geldanamycin and ATP bound to the N-terminal ATP-binding site of Hsp90.

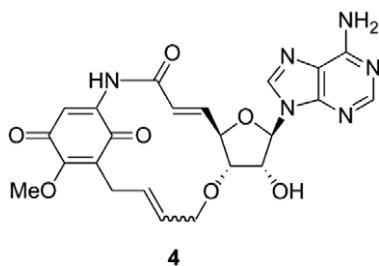
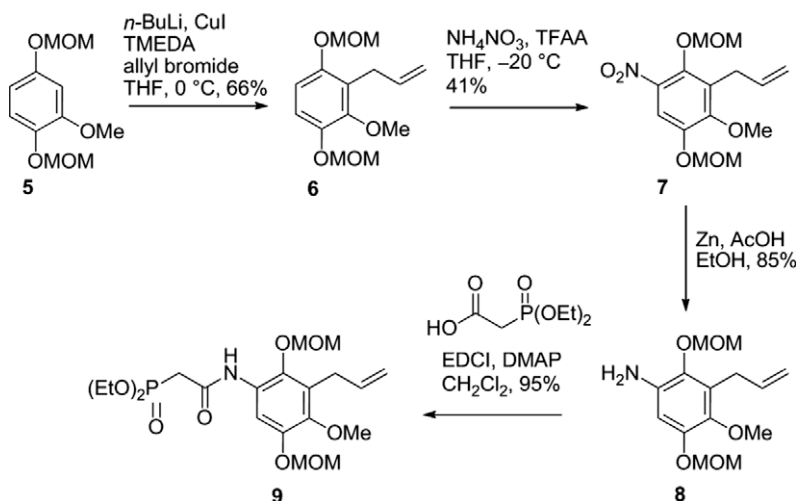


Figure 2. Structures of 3',5'-ansa-adenosine derivatives.

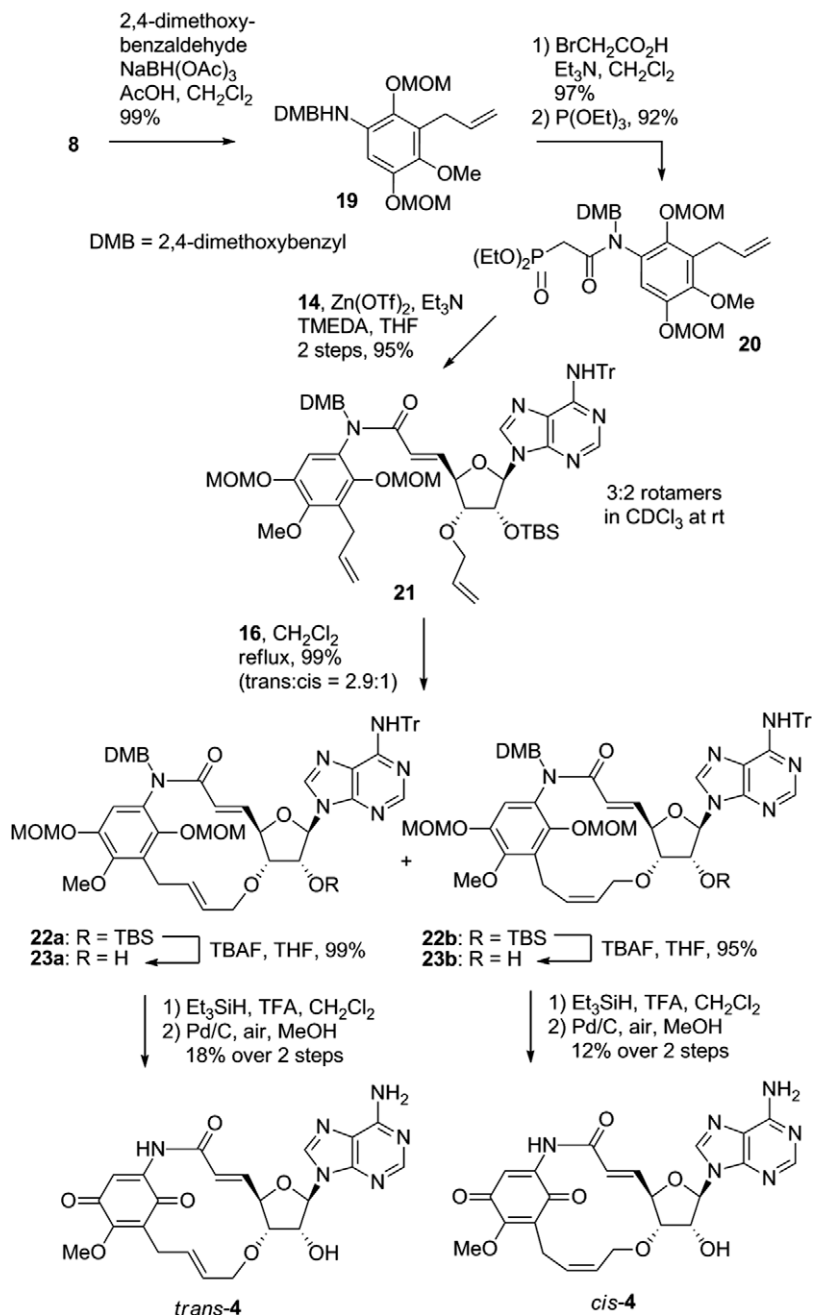
alcohol **13** in 56% yield along with recovery of **12** in 34% yield. The resulting 5'-hydroxyl group of **13** was oxidized to the aldehyde **14** by IBX. Since **14** was labile under basic conditions leading to the epimerization at the 4'-position or β -elimination, mild reaction conditions would likely be necessary for the HWE olefin-

ation. The modified HWE reaction developed by Schauer et al.¹⁷ (Zn(OTf)₂, Et₃N, TMEDA, THF) was applied, and the desired unsaturated-*E*-amide **15**, a precursor to the cyclization, was selectively obtained in good yield (88% over two steps) without any epimerization at the 4'-position.

Ring-closing metathesis (RCM)¹⁸ to provide the 14-membered cyclophane was next examined. The RCM of **15** promoted by the first generation Grubbs' catalyst **16**¹⁹ gave none of the desired cyclophane, and only a mixture of the dimers²⁰ was obtained in approximately 33% yield along with recovery of **15** in 34% yield. On the other hand, the use of the Grubbs' second generation catalyst **17**²¹ afforded only the dioxabicyclo[4.3.0]nonene derivative **18**²² in 44% yield. The conformational analysis of the RCM precursor **15** by ¹H NMR provided insight into the failure of the RCM as follows. First, the chemical shift of the amide proton was observed at δ 8.60 ppm in CDCl₃, which is lower than that of the typical *N*-aryl amide proton, and strong NOEs were observed between the amide proton and both the methyl



Scheme 1. Preparation of **9**.



Scheme 3. Synthesis of 4.

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