A Short Synthesis of the Potent Antitumor Agent (±)-Hydroxymethylacylfulvene Using an Allenic Pauson-Khand Type Cycloaddition.

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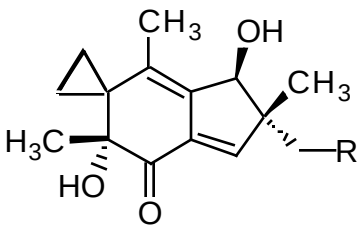
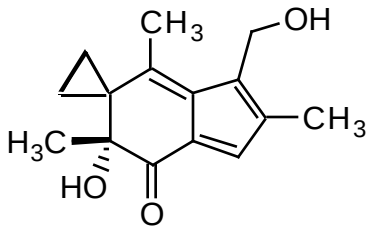
The naturally occurring sesquiterpenes illudin M (**1**) and S (**2**), have been shown to possess potent antitumor activity, but when tested *in vivo* were found to have a poor therapeutic index.[1] Subsequently, illudin analogs have been prepared that show greatly improved efficacy when compared to the parent compounds.[2] One analog in particular, hydroxymethylacylfulvene (**3**) (HMAF, also called MGI 114) has generated a significant amount of excitement since it has proven effective against breast, lung, and colon tumors in animal models while exhibiting dramatically reduced toxicity.[3] Furthermore, HMAF is effective against the MDR phenotype.[4]



(1) illudin M R = H
(2) illudin S R = OH



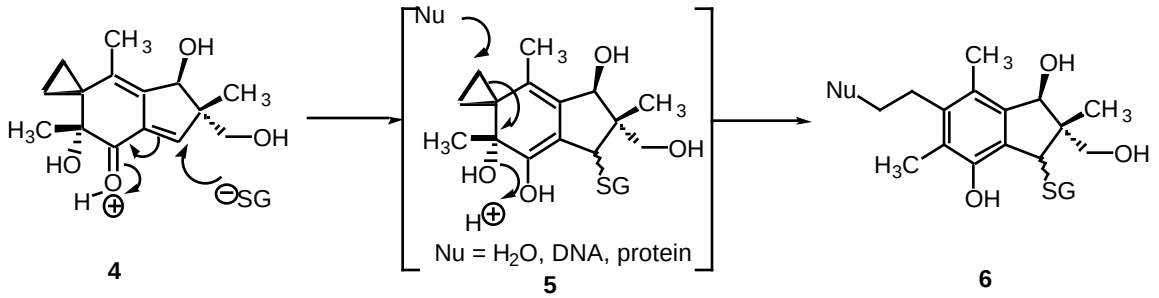
(3) hydroxymethylacylfulvene (HMAF)

HMAF (**3**) is currently in Phase II clinical trials which are being supported by the National Cancer Institute (NCI)[5] and MGI Pharma, Inc.[6] This series of Phase II trials will ultimately include studies in breast, colon, renal, ovarian, non-small cell lung, and cervical cancer. The NCI is also conducting a Phase I study in pediatric cancer patients with solid tumors. MGI Pharma, Inc. has also started to enroll patients in a Phase II study in

prostate, pancreatic and ovarian cancers. The ovarian cancer study involves women with tumors who are no longer responding or did not respond to a chemotherapy regimen that includes Taxol® and platinum-based reagents. The mechanism by which illudins selectively kill cancer cells is not well understood. Illudins bind covalently to DNA but damage induced by these agents appears to differ from that produced by other known toxins.[7]

The acylfulvene class of compounds can function as alkylating agents and this ability has been attributed to their potent cytotoxicity. For instance, McMorris *et. al*. has shown that at an optimum pH of 5.6-6.1, illudin S reacts spontaneously with sulfur nucleophiles such as glutathione (scheme 1).[1a] Glutathione adds in a Michael-type fashion to the α,β-unsaturated ketone **4** to give the very reactive cyclohexadiene intermediate **5** which is rapidly converted to the stable aromatic species **6** via a Julia-type fragmentation. It has been suggested that the nucleophiles may range from water to cellular DNA to proteins.[7b] However, in an extensive study to determine the mechanism of action of HMAF, neither interstrand crosslinks nor DNA-protein crosslinks were detected in cellular DNA.[7a]

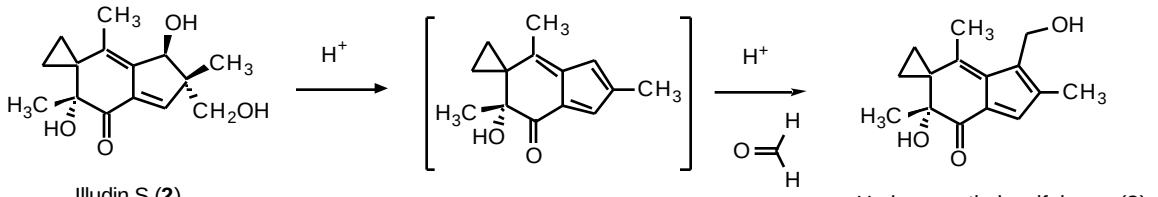
Scheme 1



4 **5** **6**

HMAF (**3**) used in these studies is obtained semisynthetically from the natural product illudin S (**2**). Illudin S is produced in cultures of *Omphalotus illudens* (Jack O' Lantern mushroom) and treatment of the illudin S with formaldehyde in 1N H_2SO_4 solution gives HMAF (scheme 2) via a reverse Prins reaction to afford the intermediate acylfulvene which then undergoes an ene reaction with formaldehyde.[1b]

Scheme 2

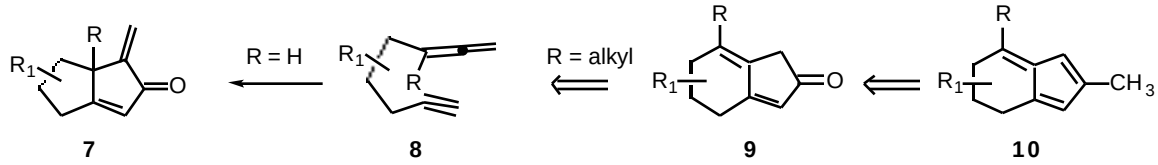


Illudin S (**2**) Hydroxymethylacylfulvene (**3**)

McMorris and coworkers[8] have reported the only synthesis of HMAF that features a Padwa-type carbonyl ylide 1,3-dipolar cycloaddition[9] to arrive at the basic illudin skeleton. We would like to report a shorter synthesis of HMAF utilizing an intramolecular [2+2+1] cycloaddition strategy developed in our group which should also permit the preparation of new analogs of the illudane class of compounds.[10]

The 3,6-dimethyl[4.3.0]nona-1,3,5-triene substructure embodied in the skeleton is unique and it was anticipated to be easily accessible by application of an allenic variant of the Pauson-Khand (P-K) type cycloaddition (scheme 3). Based upon our previous investigations, we expected that a suitably functionalized alkynyl allene **8** would cyclize to afford only **9** and none of the α-methylene cyclopentenone **7** when subjected to our standard cyclization protocol.[10a,b] The cycloadduct **9** could then be methylated and dehydrated to afford the key ring system **10**.

Scheme 3

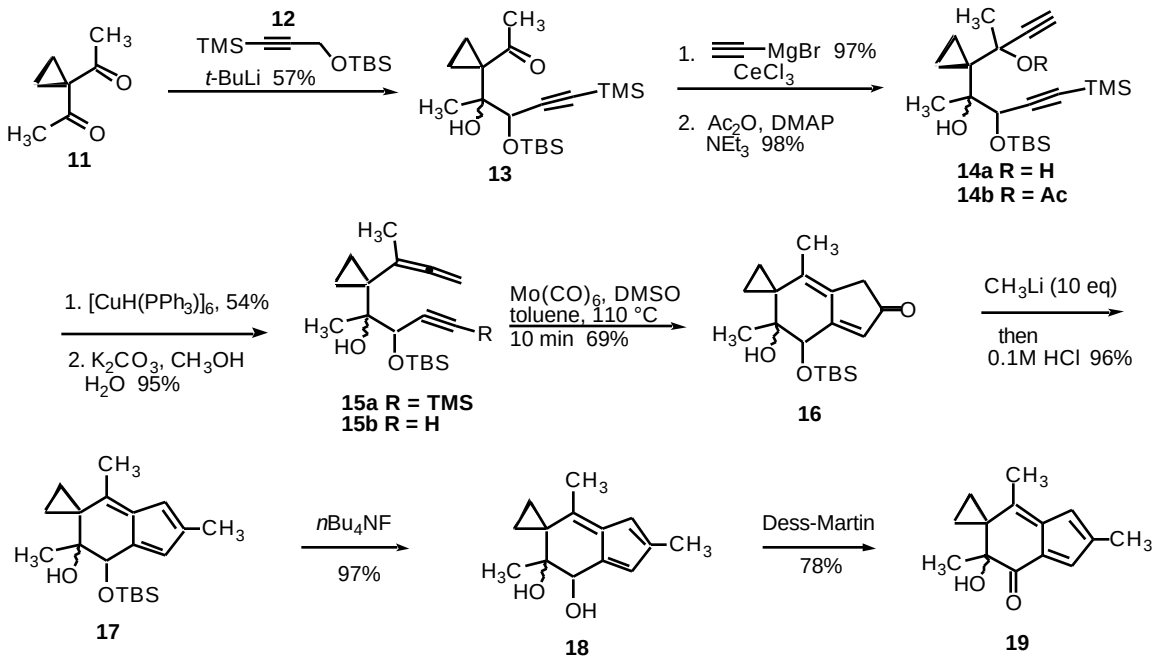


7 **8** **9** **10**

To that end, the readily available 1,1-diacetylcyclopropane (**11**),[11] was treated with the lithio derivative of the *tert*-butyldimethylsilyl ether of 3-trimethylsilylpropyn-1-ol **12** (scheme 4) to afford ketone **13** as a 1.3:1 mixture of diastereomers in 57% yield. These diastereomers were advanced through the synthetic sequence in two ways. Firstly, they were separated by column chromatography and converted to acylfulvene **19** independently, and secondly, they were taken on as a mixture to the final product, HMAF. Next, addition

of ethynylmagnesium bromide to ketone **13** in the presence of cerium(III) chloride, gave the desired propargyl alcohol **14a** in 97% yield.[12] Independent conversion of the diastereomers of ketone **13** to the propargylic alcohol **14a** showed the major isomer affording a 9.2:1 mixture of inseparable diastereomers and the minor isomer affording only one diastereomer. Next, selective formation of the propargylic acetate of the less hindered tertiary alcohol gave diyne **14b** in 98% yield. Treatment of propargylic acetate **14b** with $[CuH(PPh_3)]_6$[13] gave the allene **15a** in 54% yield. Finally, the trimethylsilyl moiety was removed from the alkyne terminus using a standard protocol to afford the desired cyclization precursor **15b** in 95% yield. We were very pleased to discover that alkynyl allene **15b** undergoes a rapid cycloaddition (10 min) under the standard allenic P-K conditions [$Mo(CO)_6$, DMSO, toluene, 110°C][10] to produce the 4-alkylidene cyclopentenone **16** as the only observed cycloadduct in 69% yield. Treatment of the ketone moiety of alkylidene cyclopentenone **16** with excess methyllithium in the presence of cerium(III) chloride gave the desired tertiary alcohol which underwent dehydration upon acidic workup to afford fulvene **17** in 96% yield. An analogous dehydration has been performed by McMorris and coworkers.[8] Removal of the TBS protecting group of the silyl ether was effected using tetra-*n*-butylammonium fluoride which provided diol **18** in 97% yield. In order to compare our synthetic material to an authentic sample, the secondary alcohol of compound **18** was oxidized to the ketone to give the acylfulvene **19** in 78% yield. The ^1H NMR spectrum of synthetic **19** was identical to the spectrum of an authentic sample provided by MGI Pharma, Inc. The synthesis of HMAF was completed using the previously reported procedure, whereby the hydroxymethyl moiety is introduced by treatment of acylfulvene **19** with paraformaldehyde and sulfuric acid in acetone/water in a 68% yield.[8]

Scheme 4



In conclusion, we have rapidly assembled the potent antitumor agent, hydroxymethylacylfulvene (**3**) in 11 steps from commercially available starting material. The synthesis features a novel application of the allenic Pauson-Khand type cycloaddition to provide a facile entry into the illudin ring system. In addition, this synthetic strategy may provide a route to analogs that are not accessible using the existing total synthesis or semisynthetic route. Efforts towards this end, are currently being investigated in our laboratories.

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Supporting Information Available: Experimental details (8 pages). See any current masthead page for ordering and Internet access instructions.

References and Notes.

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(6) Unpublished data, see: http://www.mgipharma.com/mgi/product/mgi114abs.htm

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