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THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF ENEDIYNE MINOR GROOVE BINDING HYBRIDS

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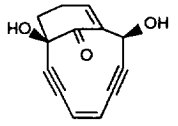
Abstract: The potential for increasing the antitumor potency of a synthetic bicyclic enediyne by attaching a DNA recognition element has been investigated and the hybrids evaluated for antitumor activity.

The natural products esperamicin and calicheamicin have attracted considerable interest from the synthetic community because of their structural novelty and their unparalleled cytotoxicity.[1] It has been shown that the oligosaccharide of these natural products binds to DNA and gives these agents their sequence specificity.[2] We have been interested in the possibility of preparing simple bicyclic enediyne hybrids which contain a simple DNA binding domain. With this strategy we had hoped to increase the potency of synthetic enediynes by increasing their affinity for DNA.

We chose to prepare hybrids of enediyne **1**, which is significantly less potent than esperamicin A_1 but has shown promising antitumor activity in various preclinical models.[3]

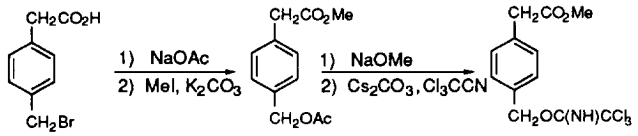


1

We sought to append a DNA recognition element that was based on the natural products netropsin and distamycin which are characterized by strong binding in the minor groove of AT rich tracts of DNA. Molecular Modeling of the natural oligosaccharide binding domains of esperamicin and calicheamicin has suggested that the role of the hydroxylamino containing sugar is that of a scaffold for positioning the other sugars to interact with the DNA.[4] With this observation to guide our design, we chose to insert a spacing unit between the enediyne and the DNA binding domain to facilitate good contact with the floor of the minor groove while preserving optimal positioning of the enediyne for DNA strand cleavage. A rigid spacer was chosen that would preserve the approximate length and directionality of the hydroxylamino sugar. Due to the instability of enediyne **1** under basic conditions a spacer that could be

attached under neutral or acidic conditions was required. Imidate **2** was ideally suited for these requirements. It contains an carboxylic ester for attachment of the groove binder via amide formation and the benzylic imidate would enable benzyl ether formation to the propargylic hydroxyl of enediyne **1** under acidic conditions. Imidate **2** was prepared from the commercially available 4-bromomethyl phenyl acetic acid according to Scheme I.

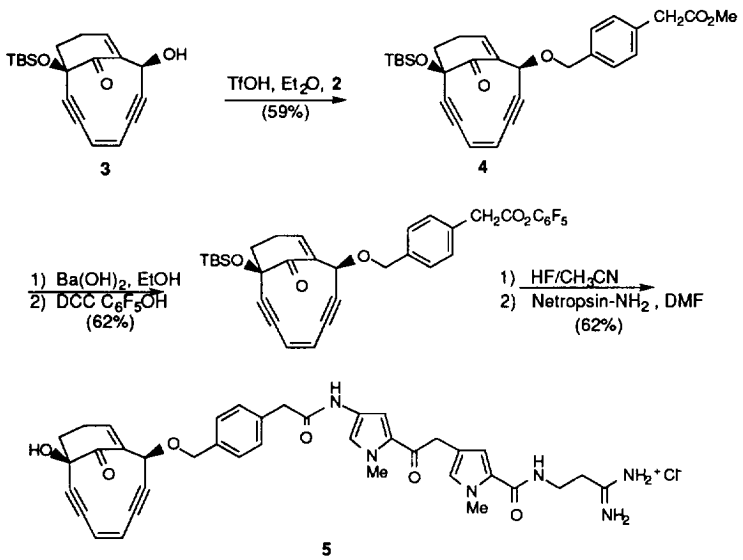
Scheme I.



2

Imidate **2** was reacted with the silyl protected enediyne **3** to give the desired benzyl ether **4**. The methyl ester was then converted to a pentafluorophenyl ester and condensed with the netropsin amine, prepared according to the method of Lown,[5] to give hybrid, **5**.

Scheme II.

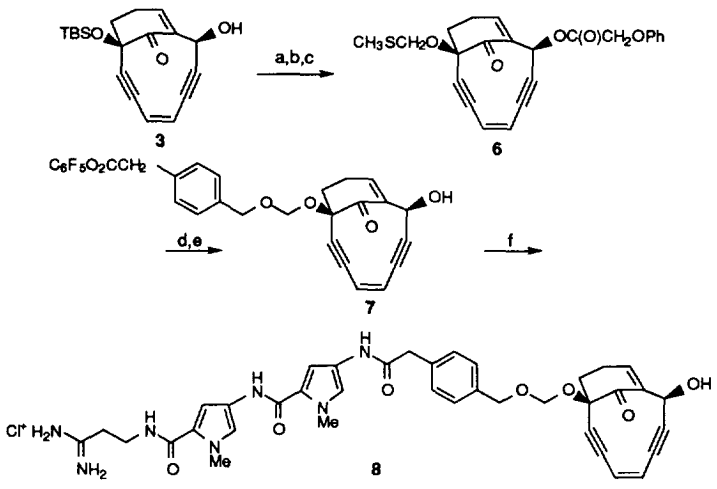


3 **4** **5**

SAR studies on enediyne **1** indicated that a free secondary propargylic alcohol was advantageous therefore a method for attaching the groove binder to the tertiary alcohol was

investigated. The MTM ether **6** was readily available from enediyne **3** in three steps. Deprotection followed by acetal exchange in the presence of the benzylic alcohol bearing the pentafluorophenyl ester provided the activated ester **7** directly. Addition of the netropsin amine then produced the second hybrid, **8**.

Scheme III.



a) $PhOCH_2COCl$ (75%); b) HF, CH_3CN (90%); c) DMSO, Ac_2O (85%); d) $Ba(OH)_2$, EtOH (73%) e) TESOTf, NIS, $F_5C_6O_2CCH_2$-Ar-CH_2OH (54%); f) Netropsin-NH_2, DMF (67%).

The hybrids were tested *in vitro* and compared to the natural product esperamicin and enediyne **1**. The DNA cleavage affinity was determined by the concentration of drug which induced cleavage of 50% of PM_2 supercoiled DNA in the presence and absence of β–mercaptoethanol. Cytotoxicity was assayed by determining the IC_{50} against the HCT-116 human colon tumor cell line.[6] The addition of the binding domain improved the DNA cleavage potency by 100 fold for **5** and 10 fold for **8** but a concomitant increase in potency against HCT-116 was not observed. Hybrids **5** and **8** were inactive *in vivo* up to the maximum tolerated dose against ip implanted M109[7] when given in a single injection 5 days after tumor implantation. These examples serve to illustrate the pitfalls of using DNA cleavage activity *in vitro* to predict antitumor activity. The cytotoxicity and *in vivo* data on the hybrids vs **1** suggests that that the cellular target of **1** may not be DNA. It has been shown that enediyne **1** damages cellular proteins while esperamicin and calicheamicin do not at cytotoxic concentrations *in vitro*.[8] Therefore targeting **1** to DNA may have destroyed activity for its

cellular target. This suggests that the oligosaccharide domain of esperamicin and calicheamicin may also function to block binding to and cleavage of cellular proteins while enhancing DNA binding and cleavage. Hybrids **5** and **8** may also suffer from low penetration into cells, a function that the oligosaccharide domain may enhance in the natural products.

Table I. Biological Activity of Netropsin Enediyne Hybrids

	PM_2 DNA cleavage	IC_{50} HCT 116
esperamicin A_1	w/ thiol $\leq 10^{-6}$M w/o thiol 10^{-5}M	1×10^{-12}M
1	w/ thiol 10^{-4}M w/o thiol 10^{-3}M	1×10^{-7}M
5	w/ thiol $\leq 10^{-6}$M w/o thiol 10^{-5}M	6.4×10^{-4}M
8	w/ thiol 10^{-5}M w/o thiol 10^{-4}M	7.4×10^{-6}M

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