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SYNTHESIS AND BIOLOGICAL ACTIVITY OF MODIFIED ENEDIYNE CHEMOTYPES

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Abstract: A series of electronically and sterically altered enediyne cores, based on esperamicin, were prepared using a cobalt mediated cyclization strategy in an attempt to improve the antitumor activity of this class of compounds. The biological activity of these analogs were then compared in both *in vitro* and *in vivo* models. These synthetic analogs were shown to be less cytotoxic than the naturally occuring esperamicin A1 yet generally effective as antitumor agents in a murine solid tumor model.

Since the discovery of the esperamicin/calicheamicin class of anti-tumor antibiotics considerable effort has been devoted to the elucidation of the mechanism of drug-DNA interaction, as well as to the preparation of highly simplified synthetic analogs.[1] In a recent communication, the synthetic enediyne analog BMS-181,130 (1), a simple mimic of the natural product esperamicin, was shown to be effective with regards to it's ability to cleave DNA and cause damage to several cellular protein isolates *in vitro*.[2]

As part of an ongoing structure-activity relationship study, it was rationalized that steric and/or electronic modifications to the enediyne moiety of 1 would slightly deactivate the propensity for cycloaromatization and diradical formation thus serving to modulate the reactivity of these systems.[3] In this line of thought, the corresponding cyclopentene-diyne (2) and the benzene-diyne (3) were constructed and evaluated for antitumor activity in both *in vitro* and *in vivo* models.


1   **2**   **3**
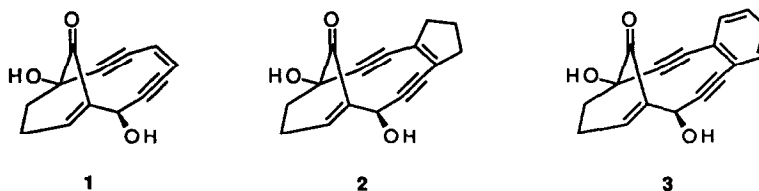
A convergent synthesis, modeled after the cobalt mediated condensation developed by Magnus and coworkers[4] and the recently reported synthesis of a precursor to 1[5], was chosen as the overall strategy for the preparation of these analogs. The enediyne containing subunits A, B, and C were prepared using a tandem palladium catalyzed coupling[6] between 1,2-dibromobenzene, dibromocyclopentene, or cis-1,2-dichloroethylene with the tetrahydropyran protected propargyl moiety, followed by a second coupling with trimethylsilylacetylene and subsequent desilylation with lithium hydroxide. Fragments A , B, and C were thus obtained in 40% , 48%, and 60% overall yield respectively as shown in Scheme 1.

Scheme 1. Generalized Synthesis of the Enediyne Fragment
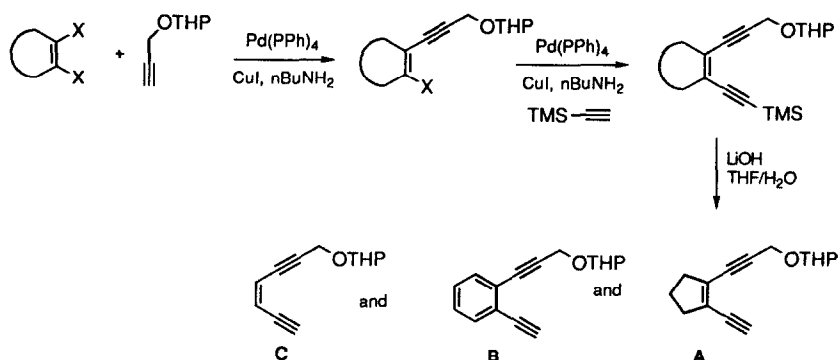
With the desired enediyne fragments in hand the analogs were then assembled in an similar manner, as illustrated in Scheme 2. Acetylene anion formation followed by condensation with the substituted cyclohexenone and trapping of the resulting enolate as the allylic carbonate smoothly furnished intermediate **D** in a one-pot reaction. The allyl enol carbonate was then converted to the enone **E** using the method described by Tsuji.[7] Removal of the tetrahydropyran protecting group and complexation of the proximate alkyne with dicobalt ocatcarbonyl gave product **F** in greater than 90% yield for all three adducts. This procedure was followed by oxidation of the propargylic alcohol to the aldehyde using 1,1'-(azodicarbonyl)dipiperidine (ADDP)[8] thus setting the stage for final ring closure and decomplexation.

Scheme 2. General Synthesis of Enediynes **2** and **3**
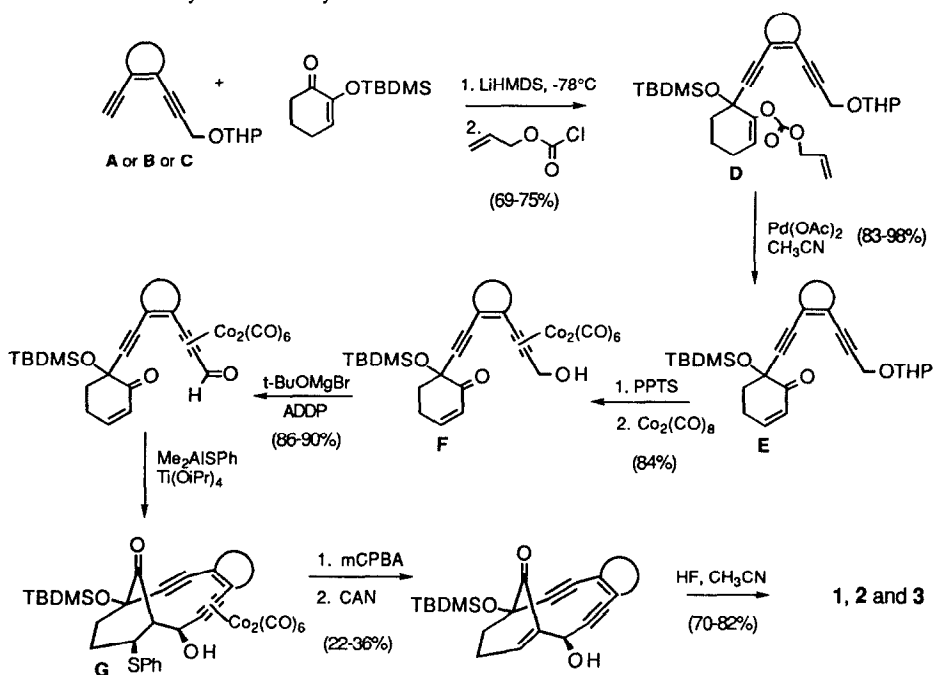
Closure to the 10-membered ring system was initiated by 1,4-addition of the aluminium phenylthiolate anion.[9] Subsequent introduction of an excess of titanium isopropoxide allowed for Aldol condensation of the resulting enolate with the propargylic aldehyde to give intermediate G in modest yields. Elimination to regenerate the desired unsaturation was accomplished by oxidation of the sulfide to the sulfoxide with m-chloroperbenzoic acid followed by removal of the cobalt complex by further oxidation with cerric ammonium nitrate. Desilylation of the tertiary alcohol provided analogs 1, 2 and 3 as crystalline solids.

The DNA cleaving and cytotoxic properties of these analogs were examined and compared to those of a naturally isolated enediyne, Esperamicin A1, as shown in Table 1. *In vitro* activity was evaluated by inducement of the SOS response[10], which is a colorimetric assay for detecting DNA-damaging agents which induce cell filamentation. The IC_{50} cytotoxicity value is reported in molar concentration and was determined in the HCT-116 human colon cancer cell line.[11] The ability of the synthetic analogs to cleave DNA was confirmed in a qualitative manner using supercoiled pM2 DNA. The asterisk notation (the number of which indicates greater activity) in Table 1 is used as an indication of relative cleavage activity in experiments conducted with and without the presence of β-mercaptoethanol (β-SH) and drug concentrations at the 2×10^{-5} M level. The *in vivo* studies were conducted using intraperitoneally (i.p.)-implanted murine Madison 109 (M109) lung carcinoma cells. Once the tumor was established, the compounds were injected i.p. at several dose levels on day 5 of the experiment.[12] The T/C values reported refer to the relative median survival times of drug-treated and control mice, x 100 (and expressed as a percentage); the optimal doses (in mg/kg) used to achieve the maximum effects are also provided. A T/C ≥ 125% was considered an active result.

Table 1. Summary of Biological Data

Compound	SOS Chromotest Dose (μg/mL)	Area (mm^2)	IC$_{50}$ (HCT-116)	pM2 DNA Cleavage	%M109 %T/C (mg/kg/inj)[a]
esperamicin A1	100	654	1.0×10^{-12} M	with β-SH ****	143-162
	20	592		no β-SH ***	(0.040-0.048)
	4	554			
1	1000	1621	1.0×10^{-7} M	with β-SH **	144 (16)
	200	954		no β-SH *	
	40	427			
2	1000	162	2.2×10^{-6} M	with β-SH *	147 (64)
	200	75		no β-SH *	
	40	0			
3	1000	0	1.8×10^{-6} M	with β-SH **	107 (64)
	200	0		no β-SH *	
	40	0			

[a] %T/C reflects increased lifespan relative to control groups ; optimal dose administered ip on Day 5 post-tumor implant (or highest dose tested if inactive, i.e., T/C < 125%) is shown in parentheses.

The simplified analogs, 1,2, and 3 were several orders of magnitude less potent than esperamicin A1. Within the simplified structural class itself, modifications of the enediyne fragment decrease the IC_{50} in HCT-116 cells by at least one order of magnitude but the compounds are still reactive with respect to their DNA cleavage ability via diradical formation, however the presence of β-mercaptoethanol is required since absence of thiol greatly reduces cleaving ability. Both analogs 1 and 2 were active *in vivo* in the M109 assay, and their levels of activity were comparable to esperamicin A1. Consistent with their relative cytotoxic potencies, the optimal dose of analog 2 was 4-fold greater than analog 1. The corresponding benzene-diyne (3) proved disappointing since it was inactive at all doses (as great as 64 mg/kg) even

though the *in vitro* results were favorable. This may indicate that this analog is either unstable *in vivo* and is consumed before reaching the tumor or that it is not activated under physiological conditions. It is worth noting that several synthetic benzene-diyne analogs of the Dynemicin class have also been recently described by Nicolaou[3] and exhibit corresponding *in vitro* activity. However, *in vivo* data for this synthetic class has not yet been reported.

In conclusion, it has been demonstrated that simple synthetic cores (1 and 2), based on the complex natural product esperamicin can be effective antitumor agents both *in vitro* and *in vivo* . However, these analogs exhibit reduced potency and require substantially higher doses to achieve the same level of efficacy as the natural product. In comparing the synthetic analogs it appears that introduction of steric constraints in the enediyne moiety decreases, in these models, both *in vitro* cytotoxicity and antitumor activity. Although all three synthetic analogs appear to cleave DNA (weakly) and are cytotoxic in the HCT-116 cell line, compound 3 does not induce the SOS response and is inactive *in vivo* at the doses tested. Further studies examining the kinetics of diradical formation for the analogs and rationalization through molecular modeling are currently in progress.

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