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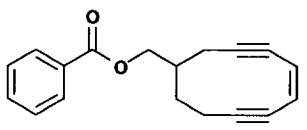
BIFUNCTIONAL ANTITUMOR AGENTS: A DNA INTERACTIVE ENEDIYNE FROM THE PYRROLO[9,10-*b*]PHENANTHRENE TEMPLATE

Graham B. Jones* and Jude E. Mathews

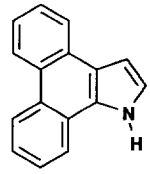
Department of Chemistry and Greenville Hospital System-Clemson University
Laboratory of Medicinal Chemistry, Clemson University, Clemson, SC 29634-1905

Abstract: Regioselective substitution of the intercalative pyrrolo[9,10-*b*]phenanthrene template was used to prepare an enediyne conjugate. The enediyne induced DNA strand scission at relevent concentration but proteolysis was not significant.

Interest in the chemistry of enediynes has grown steadily over the past decade, fuelled both by reports of their biological activity and their intriguing and challenging molecular structures.[1] In addition to total synthesis, the observed DNA cleaving activity of their cycloaromatization products (diyl radicals) has prompted development of numerous synthetic analogs and hybrids.[2] We recently developed an efficient synthetic (metallohalocarbenoid) route to linear and cyclic enediynes (e.g. **1**),[3-4] and have exploited its versatility in the synthesis of enediyne -estrogens,[5] heterocyclic enediynes,[6] and nuclear receptor substrates.[6] Guided in part by the architecture of the naturally occurring enediyne dynemicin A, we wished to design a readily accessible intercalative-enediyne hybrid to enable systematic probing of diyl radical-DNA interactions. Indeed, many classical intercalators demonstrate antitumor activity in their own right, often stemming from interaction with the DNA regulatory enzymes, topoisomerase I and II. A number of these intercalators are reported to stabilize a homodimeric complex of topoisomerase II associated to DNA, ultimately leading to protein associated DNA strand breaks.[7] Thus, harnessing a diyl radical progenitor in a ternary complex of this nature might additionally provide insight to DNA:protein interaction by investigation of proteolytic events induced by the diyl.



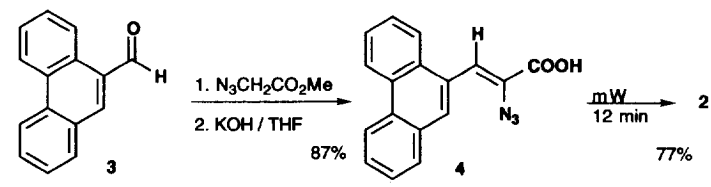
1



2

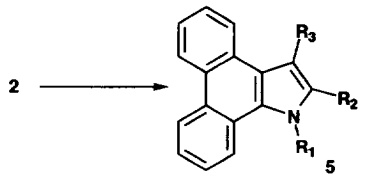
We recently reported a new intercalative template, pyrrolo[9,10-*b*]phenanthrene (**2**), and demonstrated its ability to induce intercalative unwinding of B-DNA and act as a topoisomerase poison.[8] Unsurprisingly, a β chloroethylamine hybrid derived from this system demonstrated extremely potent cytotoxicity against human colon tumor (HCT-116) cells.[8] In addition to providing a requisite vehicle for delivery of DNA interactive functionality, template **2** was designed to confer predictable indole-like chemical reactivity to the heterocyclic ring, important for future library synthesis and in any required structure - activity correlations of derivatives. Encouraged by preliminary analysis of **2**, we elected to devise a more expeditious synthesis, and demonstrate its versatility in the construction of enediyne hybrids. Commencing from commercially available carboxaldehyde **3**, the azido acid **4** was prepared, then subjected to microwave mediated thermolysis,[9-11] which produced **2** directly in good yield, *via* a tandem nitrene insertion-decarboxylation sequence (Scheme

Scheme 1



1).[12] Regioselective functionalization of **2** was then investigated employing conventional indole chemistry.[13,14] Gratifyingly, a range of functional groups were introduced with ease, including *N*-alkyl (**5a**), formyl (**5b** and **5e**), arylsulfonyl (**5c**), trialkylsilyl (**5d**), and carboxyl (**5f-g**), as shown in Scheme 2.

Scheme 2. Regioselective functionalization of pyrrolo[9,10- *b*]phenanthrene

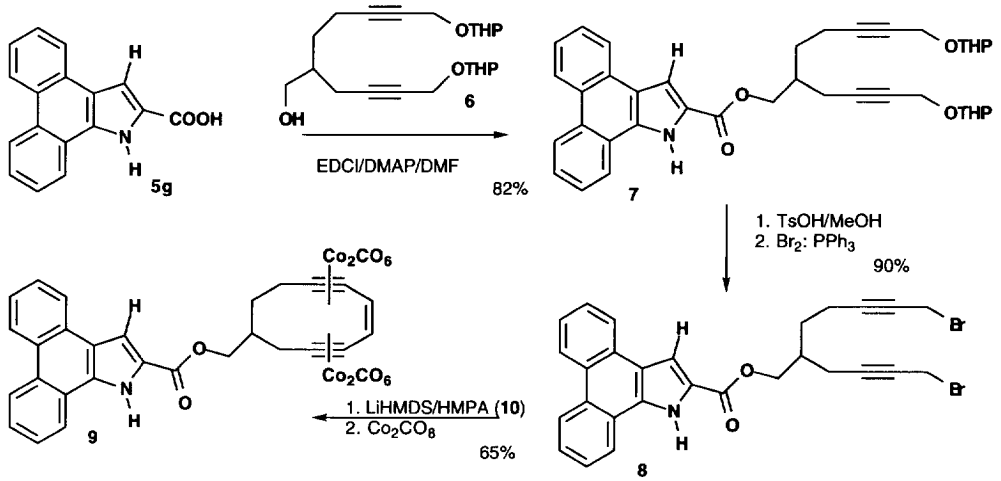


	R_1	R_2	R_3	reagents
5a	CH_3	H	H	a
5b	H	H	CHO	b
5c	SO_2Ph	H	H	c
5d	H	TMS	H	d
5e	H	CHO	H	e
5f	H	CO_2CH_3	H	f
5g	H	CO_2H	H	g

Reagents: (a) NaH, DMF then CH_3I (93%) (b) $POCl_3$, DMF (79%) (c) *n*BuLi then $PhSO_2Cl$ (99%) (d) *n*BuLi, CO_2, then *t*BuLi, TMSCl then Δ (83%) (e) *n*BuLi, CO_2, then *t*BuLi, DMF, then Δ (55%) (f) *n*BuLi, CO_2, then *t*BuLi, CH_3OCOCl, then Δ (63%) (g) as f, then LiOH, THF (95%) or *n*BuLi, CO_2, then *t*BuLi, added to CO_2, then Δ (43%).

With suitable templates in hand, we turned attention to the design of an enediyne hybrid. Based on molecular modeling considerations, we had reasoned that an effective DNA cleaving system would result from cyclization of an enediyne hybrid, such that the diyl locus was positioned approximately 10A from the mid-point for intercalation, with the tetrahydronaphthyl subunit oriented in the tract of the minor groove.[15] This arrangement would establish ideal alignment for atom transfer from the DNA backbone, resulting in strand scission.

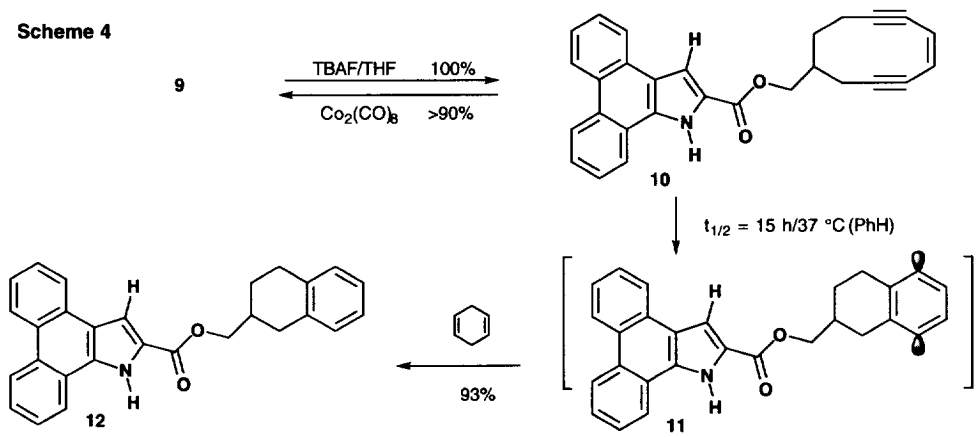
Scheme 3. Coupling of enediyne precursor to intercalative template and carbenoid cyclization



Accordingly, ester **10** was initially identified, and prepared as shown in Scheme 3. Coupling of acid **5g** with the diyne synthon **6**[5] was promoted using the conditions of Boger to give ester **7**.[16] Deprotection

followed by propargylic bromination then gave dibromide **8** in good yield, which was subjected to the carbenoid insertion-elimination process to give **10** directly.[4] This intramolecular route to the enediyne core is noteworthy for its mildness and toleration of pendant functionality, and in this particular instance it was found unecessary to protect the NH function. For practical purposes, the product enediyne was isolated as its bis-cobalt carbonyl complex **9**, providing a shelf-stable form of the enediyne, which could be unmasked at will to regenerate **10** (Scheme 4). Deprotection of **9** followed by Bergman cycloaromatization of **10** was conducted at physiological temperature in the presence of the atom transfer agent 1,4-cyclohexadiene (30 equiv.), which with a half-life of approximately 15 h, gave adduct **12** in high yield, presumably via the 1,4 diyl intermediate **11** (Scheme 4).

Scheme 4



DNA Cleavage

The ability of conjugate **10** to induce DNA strand scission was evaluated using supercoiled bacteriophage (ΦX174 RfI) DNA. Lesions were evident at agent concentrations as low as 10^{-6} M, however significant strand scission did not occur until 10^{-4} M (Table 1).[17] The predominant events involved single stranded cutting, with the onset of random single stranded cutting events at higher concentration (10^{-3} M).[18] The additional benefit of the intercalative group was confirmed however by comparison of the cleavage induced by enediyne benzoate **1**.[4] Similar observations were also made with antitumor activity against HCT-116 cells.[19]

Table 1. DNA Cleavage Induced by Enediyne Hybrid **10** and Enediyne **1**

DNA	control	10^{-5} M **10**	10^{-4} M **10**	10^{-3} M **10**	10^{-5} M **1**	10^{-4} M **1**	10^{-3} M **1**
RF I	95%	79%	45%	7%	93%	78%	15%
RF II	5%	21%	55%	88%	7%	22%	82%
RF III	0%	0%	0%	5%	0%	0%	3%

Conditions: ΦX174 RfI DNA (50 μM in base pairs) was incubated in TRIS-HCl at pH 8.5 as control or with **10**/**1** at indicated concentrations for 12 h at 37 °C. The crude mixtures were subjected to gel electrophoresis (10% agarose), stained with ethidium bromide, then subjected to scanning densitometry.

A potentially more critical target of enediynes, and the diradicals they generate, are nuclear proteins. Both the naturally occurring kedarcidin and the related enediyne neocarzinostatin, are chromoproteins that are capable of causing protein damage to histones,[20] and the Bristol-Myers-Squibb group has also described the proteolytic activity (10^{-4} M) of a synthetic ten-membered enediyne.[21] Accordingly, the proteolytic activity of **10** was assessed using histones II-S, II-AS, and III-S, but evidence of agglomeration was not detectable until

10^{-3} M, suggesting the primary target of the pyrrolo[9,10-*b*]phenanthrene hybrid may indeed be DNA, as predicted.

Conclusion

A three step route to the intercalator pyrrolo[9,10-*b*]phenanthrene has been accomplished, and the synthetic utility of this template has been demonstrated. Preliminary bioassay of an enediyne conjugate suggests DNA interaction to be favorable, and that strand scission can be induced at low concentration.

Acknowledgments

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