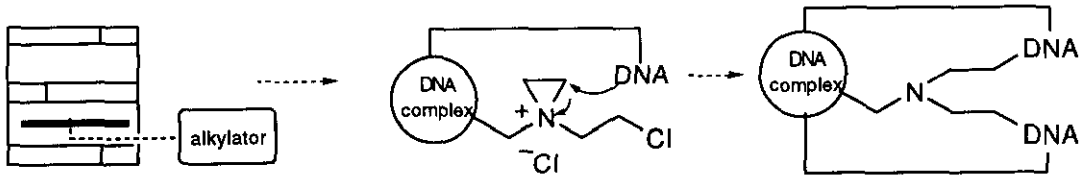
BIFUNCTIONAL ANTITUMOR AGENTS: PYRROLO-[9,10]PHENANTHRENES AS INTERCALATIVE DRUG DELIVERY VEHICLES

Graham B. Jones,* Jude E. Mathews, and W. Kenneth Banner

Howard L. Hunter Laboratories, Department of Chemistry,
Clemson University, Clemson, SC, 29634-1905, USA

Abstract - The preparation of a pyrrolo[9, 10]phenanthrene nucleus is described. The utility of the template as a potential DNA intercalating drug delivery system is demonstrated by synthesis of a derived bis chloroethylamine alkylating system.

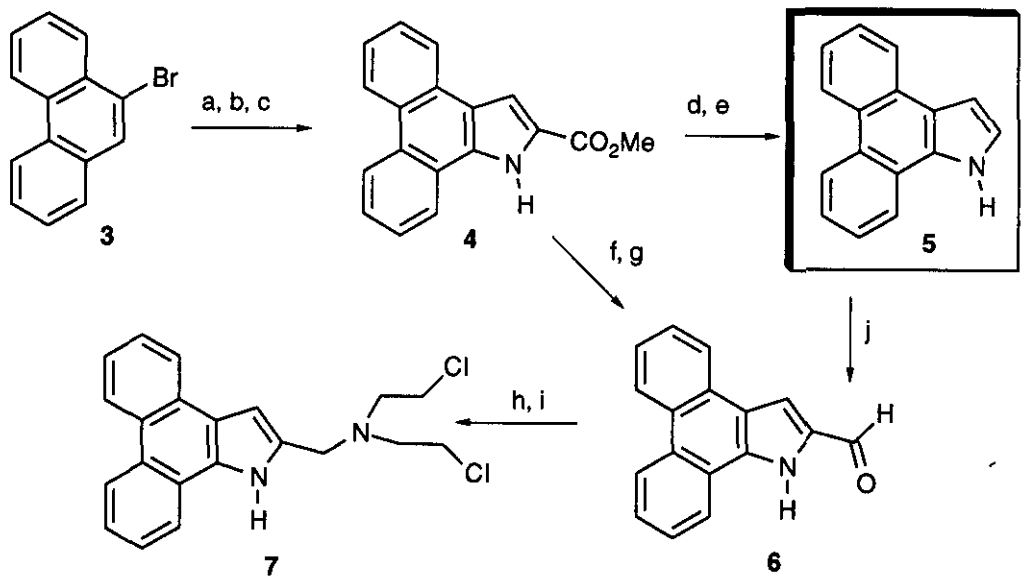
Agents with the capacity to form covalent links to duplex DNA have proven useful both in cancer chemotherapy and in the elucidation of nucleic acid reactivity.[1] A number of bisalkylative nitrogen mustard agents are currently under investigation as potential antineoplastic agents, a result of both their high reactivity and the presumed biological consequences of DNA cross linking.[2] As part of a program directed toward synthesis of antitumor agents, we envisioned that coupling of a heterocyclic derived delivery template which could effectively intercalate DNA with a bis alkylative mustard agent, would result in synergistic enhancement of DNA immobilization (Scheme 1). The (reversible) binding of such a species would deliver the reactive β chloroethylamine moieties to



intercalated DNA-drug complex DNA alkylation DNA crosslinking

Scheme 1

their target, thus allowing single and double DNA alkylation via aziridinium ion formation and subsequent nucleophilic attack by a proximal base. This could encompass inter and intrastrand alkylation, and modification of the intercalative template may allow discrimination in DNA binding selectivity, a possible application thereafter being gene specific DNA inactivation. For this reason, the (intercalative) delivery template must be flexible, easily functionalised and satisfy the criteria for efficient binding. We herein report the synthesis of a new heterocyclic delivery system based on pyrrolo[9,10]phenanthrene (**5**) and its efficiency in DNA immobilization. The choice of the pyrrolophenanthrene group as a delivery vehicle is supported by a variety of factors: (i) the generally accepted requirement of three or more fused coplanar aromatic rings for efficient intercalation to DNA.[3] (ii) the expected high chemical reactivity of such a template, allowing subsequent functionalization and modification of the pyrrole ring using established methods. (iii) molecular modeling of derived systems, showing ideal positioning of appendages for productive covalent and non covalent interactions.[4] The route secured to synthesize the parent



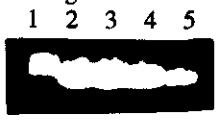
Reagents: (a) BuLi, DMF, 95% (b) $N_3CH_2CO_2Me$, NaOMe, 94% (c) Δ / 140°,92% (d) LiOH-THF, 97% (e) microwave thermolysis[7], quinoline, 89% (f) $LiAlH_4$, THF, 98% (g) MnO_2, CH_2Cl_2, 79% (h) $HN(CH_2CH_2OH)_2$ / $NaCNBH_3$, 71% (i) $SOCl_2$ / DMF, 88% (j) BuLi / CO_2 / BuLi / DMF, 55%[12]

Scheme 2

unsubstituted chromophore (**5**) is illustrated in Scheme 2.[5] Formylation of 9-lithiophenanthrene was followed by conversion to an azidocinnamate, using the established Rees-Moody protocol.[6] Thermolysis of the azide in

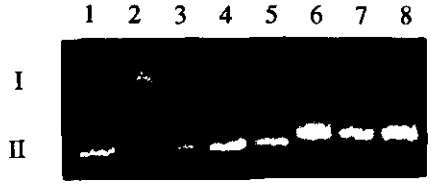
refluxing xylenes gave the tetracyclic ester (**4**) in high yield. Conversion to the desired unsubstituted parent system (**5**), using a microwave accelerated decarboxylation of the derived acid, proceeded in nearly quantitative yield.[7] Confirmation of the structure (**5**), was achieved by 2D COSY nmr, with characteristic coupling between the indolyl NH and C2/C3 protons evident.[8] The propensity for the parent pyrrolophenanthrene (**5**) to participate in intercalative processes with DNA was then examined. In the uv spectral analysis of a mixture of **5** and calf thymus DNA, characteristic red shifts of 10 nM at 290 nM for DNA were observed on incubation, consistent with intercalation.[3] Furthermore, incubation followed by electrophoretic examination of a mixture of **5** + ΦX174 Type I DNA showed characteristic streaking of the supercoiled species, indicative of intercalation (Figure 1, lanes 2-4).[9] A second assay was performed to confirm the existence of an intercalated complex. Conversion of type I to type II DNA, accelerated by the regulatory enzyme topoisomerase I can be surpressed in the presence of intercalators.[10]

Figure 1.



ΦX174 supercoiled (Type I) DNA incubated with agent for 18 h at 24°C in 10 mmol Tris-HCl, 1 mmol EDTA, pH 8.0 (total volume 6 µl) followed by gel electrophoresis (1% agarose, ethidium bromide stain). Lane 1= DNA (50 µmol) - ethidium bromide (50 µM); Lanes 2-4 = DNA (50, 100, 250 µmol respectively) - **5** (50µmol); Lane 5 = DNA (50 µmol)

Figure 2.



Inhibition of DNA topoisomerase induced relaxation by **5**. ΦX174 DNA (Type I) incubated (30 min, 24°C) in Tris-HCl at pH 7.5 with agents (total volume 7.3 µl) followed by gel electrophoresis (1% agarose, ethidium bromide stain). Lane 1 = DNA (50 µmol); Lane 2 = DNA (50 µmol) - topoisomerase I (1 µl: 8 units / µl); Lanes 3-5 = DNA (50 µmol), topoisomerase I (1 µl. 8 units / µl) and 50, 250 and 100 µmol of **5** respectively. Lanes 6-8 = DNA (50 µmol), topoisomerase I (1 µl: 8 units / µl) and 50, 250 and 100 µmol of ethidium bromide

Incubation of DNA with topo I in the presence of **5** however resulted in inhibition of relaxation at 250 µmol (Figure 2, lane 4). Having confirmed the ability of the parent tetracycle (**5**) to associate with DNA, we sought to demonstrate its capacity to deliver a reactive alkylative species to such a target. Transformation of ester (**4**) to carboxaldehyde (**6**) proceeded without incident, allowing a reductive amination strategy to introduce the important β hydroxyethylamine function (Scheme 2). Finally, derivitization of the diol to form the alkylating system (**7**) was accomplished. Preliminary antitumor evaluation of **7** confirmed the potency of the bis chloroethylamine appendage with good activity in the Human Colon Tumor assay employed.[11] Comparison with the observed IC_{50} values for related mustard systems supported the design strategy behind the bifunctional system and the inclusion of an intercalative moiety.[11] This rationale is supported by molecular modeling performed on **7** which confirmed the possibility of DNA cross linking, both interstrand and intrastrand.[4] Future studies directed towards isolation of drug:nucleotide adducts, in tandem with structural modifications to the

intercalative template are designed to reveal the exact nature of the DNA intercalation-alkylation process. As expected, the chemical reactivity of template (**5**) parallels indole, allowing a range of functional groups to be introduced directly at the nitrogen, C3 position, and via the Katritzky procedure, at the C2 position.[12] It is anticipated that such substituted pyrrolophenanthrenes will find a variety of related applications where reversible binding to DNA is desired.

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