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SYNTHESIS AND DNA CROSSLINKING ABILITY OF A DIMERIC ANTHRAMYCIN ANALOG

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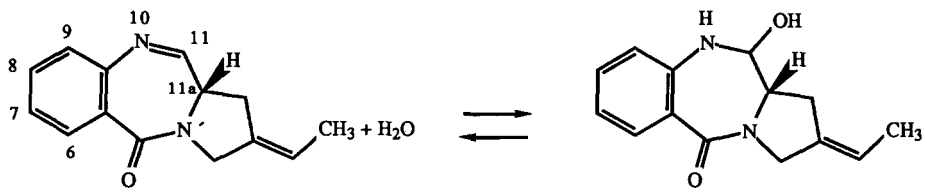
Summary. Linked analogs of the DNA binding antibiotic anthramycin are made via nucleophilic aromatic substitution followed by reduction-cyclization. The linked compounds protect DNA from restriction endonucleases and reversibly crosslink DNA.

The antitumor antibiotics of the anthramycin family (pyrrolo[1,4]benzodiazepines) have been shown to bind covalently and reversibly at their 11-position (cf. **1**) to the NH_2 of guanine located within the DNA minor groove.[1] Since this class of compounds binds exclusively to duplex DNA[2] (and not to single stranded DNA, proteins, RNA or RNA-DNA hybrids), it appeared that a molecule with two anthramycin moieties linked by a flexible tether would exhibit novel DNA crosslinking properties. Furthermore, many active antitumor agents work by crosslinking DNA.[3]

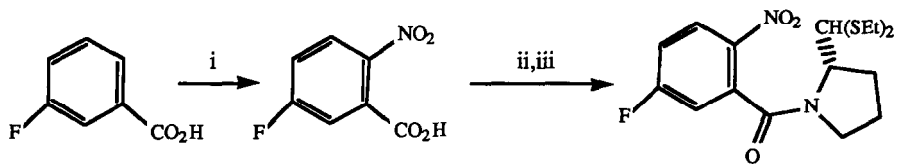
One problem to be overcome in the synthesis of anthramycin derivatives is the imine moiety. Under physiological conditions, the imine is in equilibrium with a carbinolamine (illustrated by prothracarcin (**1**)). This functionality is chemically labile; therefore, vigorous conditions can induce decomposition. Also,



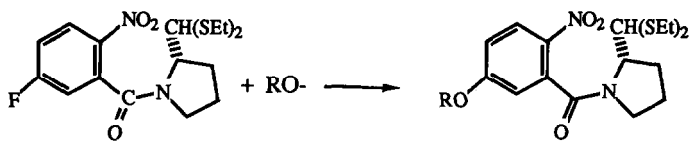
(1)

epimerization at carbon 11a is to be avoided, since the 11a-R epimer does not bind to DNA.[4] It thus appeared prudent to link together pyrrolo[1,4]benzodiazepine precursor units prior to the generation of the imine. Nucleophilic aromatic substitution effected this linkage in excellent yield, leading to the first synthesis of linked anthramycin analogs. In addition, the methodology should be extendable to link anthramycin analogs to other classes of DNA binding drugs.

Nitration of *m*-fluorobenzoic acid at 0°, followed by conversion to the acid chloride and coupling with (2S)-pyrrolidine-2-carboxaldehyde diethylthioacetal[5,6] gave the amide **2**[7] in 75% overall yield. Reaction with the sodium salts of water, methanol or N,N-dimethylethanolamine with **2** in THF give the substitution products **3-5** in high yield. (Conditions: i. H_2SO_4/HNO_3, 0°, 91%; ii. oxalyl chloride/DMF; iii. THF (2S)-pyrrolidine-2-carboxaldehyde dithioacetal, 85%; iv. Pd/C-$NaBH_4$, 92%; v. $HgCl_2$, $CaCO_3$, THF/H_2O 40-85%)[8,9]



(2)

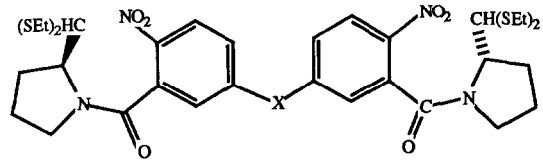


(2) R = H(**3**), CH_3(**4**), $Me_2N(CH_2)_2$(**5**)

In a similar manner, the linked compounds **10-12** were prepared from the sodium salts, except for **12**, which was prepared using the free amine.[10] Following a modification of the procedure of Langley and Thurston,[9] **3-5** and **10-11** were cyclized via reduction of the nitro group to the amine with $NaBH_4$-Pd/C, followed by deprotection with Hg^{+2} and spontaneous cyclization to the imine.[11] Overall yields of chiral imines exceeded 30% from **2**. Under a variety of reduction-cyclization conditions **15** was not formed, only decomposition products resulted. More

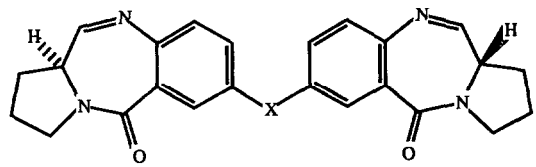
stucturely complicated nucleophiles could also be added to **2**. For example, reaction of 2,3,4,6-tetra-O-benzylglucopyranose with **2** in the presence of NaH gave the aryl glycoside, with the β-anomer as the only isomer. During the substitution, the Ar-NO_2 was reduced to the amine.

(2) + $HS(CH_2)_6SH$
+ $CH_3N[(CH_2)_2OH]_2$
+ $CH_3N[(CH_2)_3NH_2]_2$



(10) X = $S(CH_2)_6S$
(11) X = $O(CH_2)_2N(CH_3)(CH_2)_2O$
(12) X = $HN(CH_2)_3N(CH_3)(CH_2)_3NH$

(10), (11), (12) —iv,v→



(13) X = $S(CH_2)_6S$
(14) X = $O(CH_2)_2N(CH_3)(CH_2)_2O$
(15) X = $HN(CH_2)_3N(CH_3)(CH_2)_3NH$

Compound **14**, like anthramycin itself,[12] protected DNA from restriction enzyme digestion. Incubation of **14** (250 μM) and supercoiled plasmid DNA (pRWAT 14.1)[13] for 12 hours at 65°, followed by EtOH precipitation and digestion with Hinf I gave no DNA cutting. Partial protection took place with concentrations of **14** as low as 50 μM.

The crosslinking ability of **14** was examined using an alkaline agarose gel assay under the conditions which it protected the DNA.[14] Figure **1** shows linear plasmid pRWAT 14.1 DNA (lane 1) with psoralen-crosslinked DNA in lane 3 as a control. Lane 2 is DNA crosslinked with **14** (65°, 1 hour). Binding of anthramycin to DNA has been reported to retard gel mobility,[12] which may be why the **14**-crosslinked DNA runs more slowly than the psoralen-crosslinked DNA. However, linear DNA with bound anthramycin runs with uncrosslinked DNA on an alkaline agarose gel. The absence of single-stranded DNA in lane 2 shows **14** binds across the duplex rather than intrastrand, as suggested by models. Finally, the reversibility of binding was established by incubating a portion of the DNA in lane 2 at pH 10 for 12 hours, 25°, which gave a mixture of crosslinked and single-stranded DNA. Some

dimeric pRWAT 14.1, which is a contaminant, is seen in lane 4 as well. Compound **14** is the only known example of a molecule which can covalently crosslink DNA and be removed by a simple pH change.

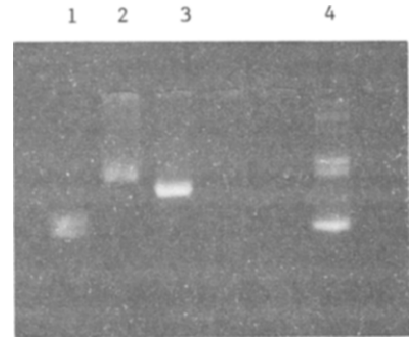


Figure 1. Alkaline agarose gel[14] of linearized pRWAT14.1 (1); linearized pRWAT14.1 crosslinked with **14** (2); the linear DNA crosslinked with psoralen (3); lane 2 DNA after dialysis against pH 10 citrate buffer, 12 hours, 25° (4).

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7. $[\alpha]_D^{23}$ (C. 0.013) -250.2. All rotations are measured in chloroform.
8. **3** $[\alpha]_D^{23}$ (0.006) -134.8; **4** $[\alpha]_D^{23}$ (0.048) -162.7; **5** $[\alpha]_D^{23}$ (0.009) -162.5
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10. **10** $[\alpha]_D^{23}$ (0.013) -250.2; **11** $[\alpha]_D^{23}$ (0.015) -158.3; **12** $[\alpha]_D^{23}$ (0.025) -173.3. **12** was prepared by reaction in isopropanol at 75° of **2** and **9.**
11. **13** $[\alpha]_D^{23}$ (0.008) +285.6; **14** $[\alpha]_D^{23}$ (0.005) +675.
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