The Synthesis and Characterization of a Series of Bis-intercalating Bis-anthracyclines

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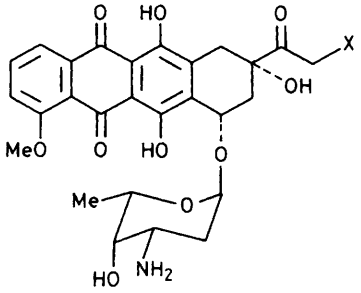
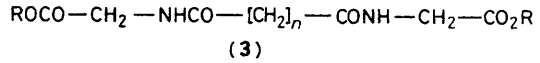
The synthesis is reported of the bis-daunomycin derivatives (**5a—g**) which have been shown conclusively to act as bis-intercalating agents of DNA and also to exhibit slow DNA-dissociation kinetics.

Lerman first recognized the mono-intercalation of DNA by small planar aromatic molecules.[1] More recently, the synthesis and evaluation of bis-intercalating compounds as new ligands for DNA with increased specificity and affinity has been addressed.[2] Dervan showed that the binding constant of a bis(methidium)spermine for DNA is 10^6 times greater than that of the monomeric compound ethidium bromide and that the dimer is about 50 times more selective.[3]

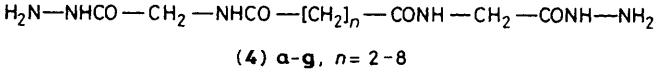
The anthracyclines daunomycin (**1**) and adriamycin (**2**), which bind to DNA by intercalation, are used clinically as anti-tumour drugs but because of their associated cardiotoxicity, total drug dosage is limited.[4] Previous reports on the synthesis of bis-anthracyclines, which had as objectives the preparation of more selective and effective drugs, include some α,ω-dicarboxylic acid hydrazones,[5] bis(thia-adriamycin) derivatives of α,ω-alkanedithiols,[6] and a bis-hydrazone derivative of 4-demethoxydaunomycin.[7] We consider that these compounds show a number of limitations including poor solubility (due to the presence of long polymethylene chains), short linker chains (which do not enable an excluded-site mode of binding[8]), nor do these reports contain good evidence to show that any of the derivatives act as true bis-intercalating agents. We now describe the synthesis, characterization, and biophysical investigation of a series of bis-daunomycin derivatives (**5a—g**).



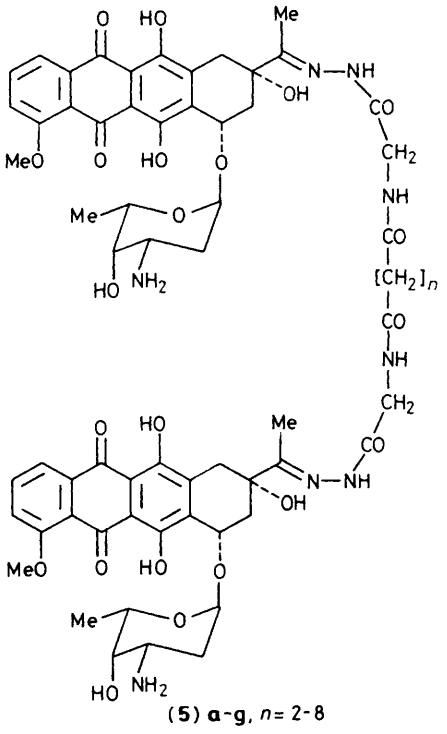
(**1**); X = H
(**2**); X = OH

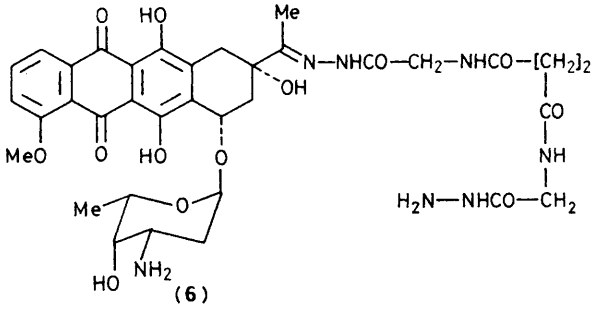


(**3**)
a; R = Bu^n, n = 2
b-**g**; R = Et, n = 3–8



(**4**) **a**-**g**, n = 2–8





The α,ω-dicarboxylic acids, glutaric acid to sebacic acid, in turn were treated with ethyl chloroformate [2.1 equiv; tetrahydrofuran (THF), Et_3N, −45 °C, N_2, 30 min]. Ethyl glycinate hydrochloride (Et_3N, H_2O, 0 °C) was added to the appropriate mixed anhydride. After the addition of 2 M K_2CO_3 and extraction with ethyl acetate, crystallization of the products from CH_2Cl_2–C_6H_{14} gave the diester diamides (**3b**—**g**).† Compound (**3a**)† was prepared from succinoyl chloride and butyl glycinate. The diester diamides (**3a**—**g**) were treated with excess of hydrazine hydrate at room temperature to produce the diacid hydrazides (**4a**—**g**).†

Each of the dihydrazides (**4a**—**g**) (0.01 mmol; MeOH–H_2O) and daunomycin hydrochloride (**1**)·HCl (0.02 mmol) were kept at 25 °C for 1—3 days. The course of the reaction was monitored by h.p.l.c.[9] [MeOH–H_2O (60 : 40), NH_4HCO_3, C_{18} reversed-phase column]. Isolation of the products and purification by preparative h.p.l.c. gave the bis-hydrazones (**5a**—**g**) in 60—80% yields (5% HCl gave the bis-hydrochloride salts). Fast-atom bombardment (f.a.b.) mass spectrometry (glycerol formic acid matrix) showed the expected $M^+ + 1$ ions at the calculated values (*m/z* 1279—1363). The ^1H and ^{13}C n.m.r. spectra of compound (**5a**) confirmed its structure.

† Satisfactory spectral data and elemental analyses were obtained.

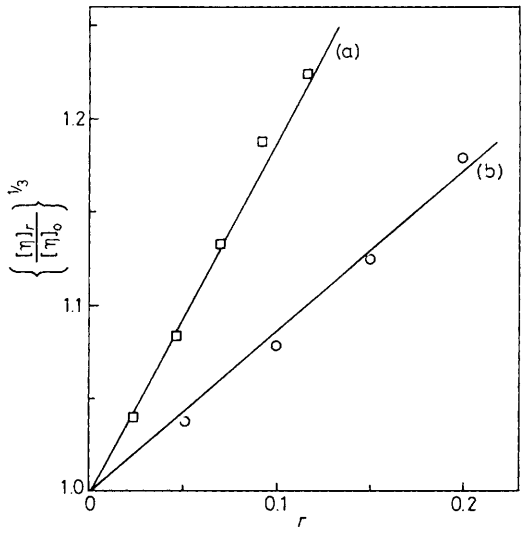


Figure 1. Viscometric titration of sonicated calf-thymus DNA (0.61 mM base-pairs) with (a), compound (**5a**), and (b), daunomycin (**1**), at 20 °C, PIPES buffer (ionic strength 0.1 M), pH 6.8. $[\eta]_r$ and $[\eta]_0$ are the intrinsic viscosities in the presence and absence of drug and *r* is the drug/base-pair ratio.

A reaction between (**1**)·HCl and (**4a**) (mole ratio, 1 : 10) produced a good yield of the mono derivative (**6**), whose structure was confirmed by ^1H, ^{13}C n.m.r., and f.a.b. mass spectral data. We did not observe rapid disproportionation of the mono adducts as had been noted with some acridine-derived intercalators.[10]

The viscosities of buffered solutions of sonicated calf-thymus DNA were determined in the presence of increasing amounts of compounds (**5a**—**g**).[11] For example, Figure 1 shows the experimental results obtained for both daunomycin (**1**) and (**5a**); the observed slope of the curve for (**5a**) is twice that for (**1**). As the helix-extension of the DNA rods caused by intercalating ligands is directly related to the viscosity observed, the results obtained are consistent with *both* chromophores of compound (**5a**) simultaneously intercalating with DNA. Similar results were obtained for all other derivatives (**5b**—**g**).

Sodium dodecyl sulphate (SDS)-induced dissociation[12] of the complexes between calf-thymus DNA and each of (**5a**—**g**) was studied to establish their dissocation rates relative to daunomycin. A solution of 10% SDS (0.5 ml, PIPES buffer, pH 6.8, ionic strength 0.01 M) was added to a solution (2 ml) of calf-thymus DNA and each of (**5a**—**g**) (drug : base pairs, 1 : 5) in the same buffer at 20 °C. The absorbance increase at 480 nm was monitored over several hours, and a simulation program CONSAM[13] was then used to analyse the data obtained. The experimental curve for the dissociation could best be described by two exponential first-order relaxation processes. The time constant for the slower process for all compounds (**5a**—**g**) varied from 430 to 1800 s; for daunomycin (**1**) a value of 0.36 s was observed. Consequently, the dissociation events for (**5a**—**g**) are 1000—5000 times *slower* than for daunomycin itself. These results are also consistent with a bis-intercalating mode of binding of (**5a**—**g**) to DNA.

Work is in progress on the cell-uptake and continuous and pulse-exposure growth-inhibition assays using L1210 cells.

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References

1 L. S. Lerman, *J. Mol. Biol.*, 1961, **3**, 18.
2 E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring, 'The Molecular Basis of Antibiotic Action,' 2nd edn., Wiley, London, 1981, pp. 258—401.
3 P. B. Dervan and M. M. Becker, *J. Am. Chem. Soc.*, 1978, **100**, 1968; M. M. Becker and P. B. Dervan, *ibid.*, 1979, **101**, 3664.
4 F. Arcamone in 'Topics in Antibiotic Chemistry,' ed. P. G. Sammes, Ellis Horwood, Chichester, 1978, vol. 2, p. 99.
5 D. W. Henry and G. L. Tong, U.S. Pat., 1978, 4,112,217.
6 R. Seshadri, M. Israel, and W. J. Pegg, *J. Med. Chem.*, 1983, **26**, 11.
7 K. M. Dawson, *Cancer Res.*, 1983, **43**, 2880.
8 G. Dougherty and W. J. Pigram, *C.R.C. Critical Rev. Biochem.*, 1982, **12**, 103.
9 P. A. Scourides, R. T. C. Brownlee, D. R. Phillips, and J. A. Reiss, *J. Chromatogr.*, 1984, **288**, 127.
10 E. S. Canellakis, Y. H. Shaw, H. E. Hanners, and R. A. Schwartz, *Biochim. Biophys. Acta*, 1976, **418**, 277.
11 W. D. Wilson, R. A. Keel, R. J. Jones, and C. W. Mosher, *Nucleic Acids Res.*, 1982, **10**, 4093.
12 W. Mueller and D. M. Crothers, *J. Mol. Biol.*, 1968, **35**, 251.
13 R. C. Boston, P. C. Greif, and M. Berman, *Comp. Prog. Biomed.*, 1981, **13**, 111.