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DNA-BINDING ABILITY OF NON-DIYNENE CLASS OF DYNEMICINS AND *AZA*-ANTHRAQUINONES

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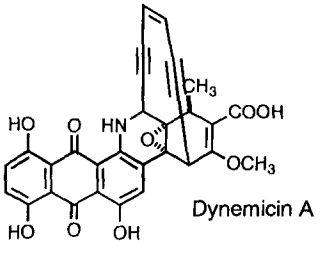
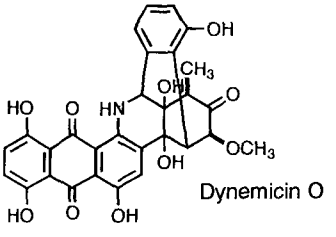
Abstract: DNA-binding ability of five non-diynene dynemicins and four related synthetic *aza*-anthraquinones (**1-4**) was studied by measurements of UV absorption shifts and electrophoretic behavior of the complexes with DNA.

Dynemicin A is a potent antibacterial and antitumor antibiotic having a striking hybrid structure combining the characteristics of both anthraquinone as a DNA intercalator and diynene as a DNA strand breaker.[1] Nevertheless, non-diynene dynemicins, dynemicins O, P and Q, produced by *Micromonospora chersina* M956-1 strain retain potent cytotoxicity comparable to that of anthracycline antibiotics.[2] Sugiura and co-workers reported on the intercalative ability of dynemicin H, a Bergman rearrangement product of dynemicin A.[3] Here we report on the DNA-binding properties of five non-diynene dynemicins and of several related tri- and pentacyclic *aza*-anthraquinones (**1-4**)[4, 5] synthesized in the course of our study to clarify the DNA-intercalative function of the anthraquinone substructure in dynemicin A.

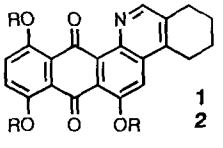
Figure 1



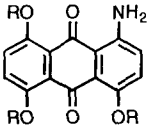
Dynemicin A



Dynemicin O

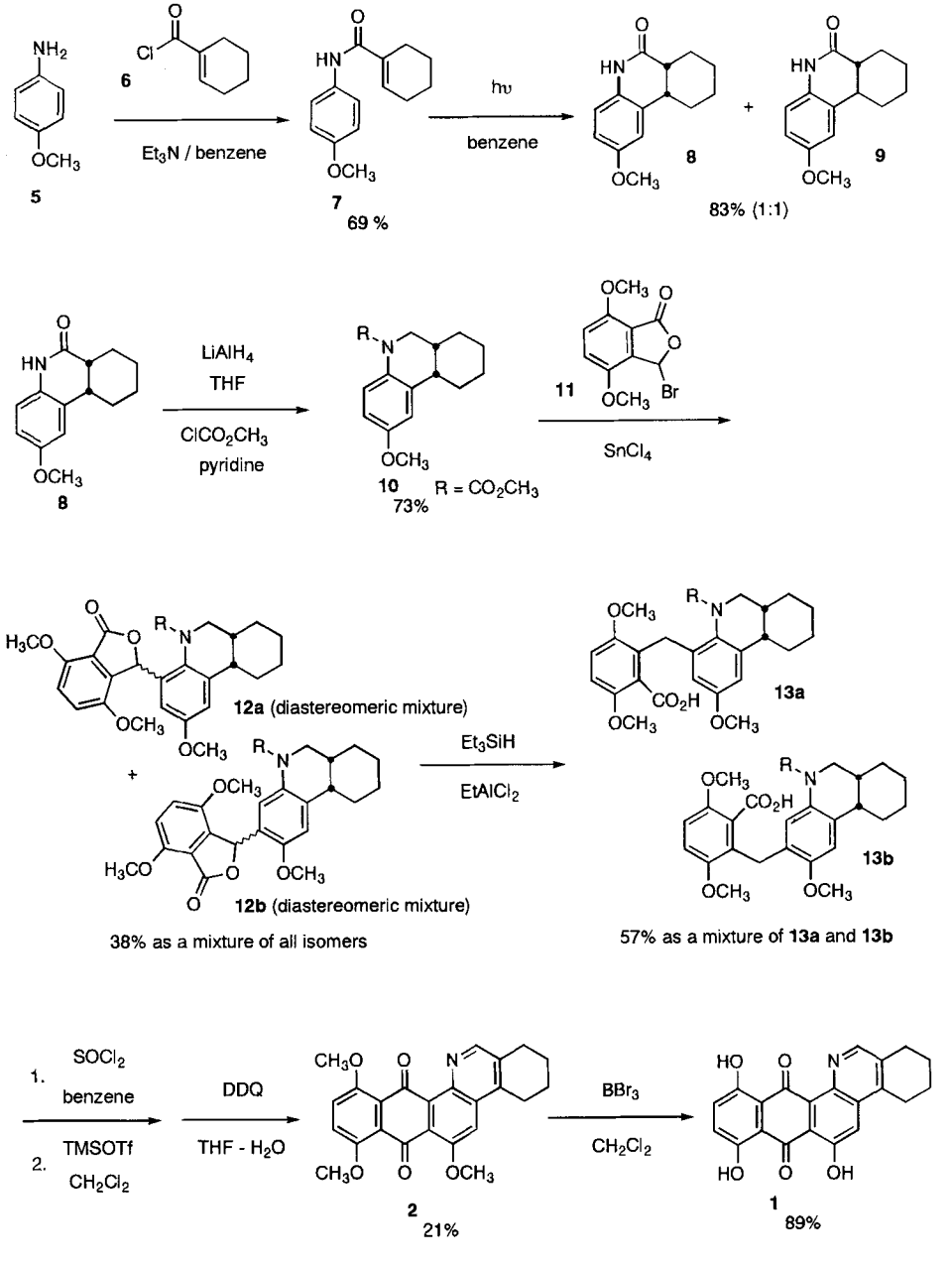


1 R = H
2 R = CH_3



3 R = H
4 R = CH_3

Scheme 1

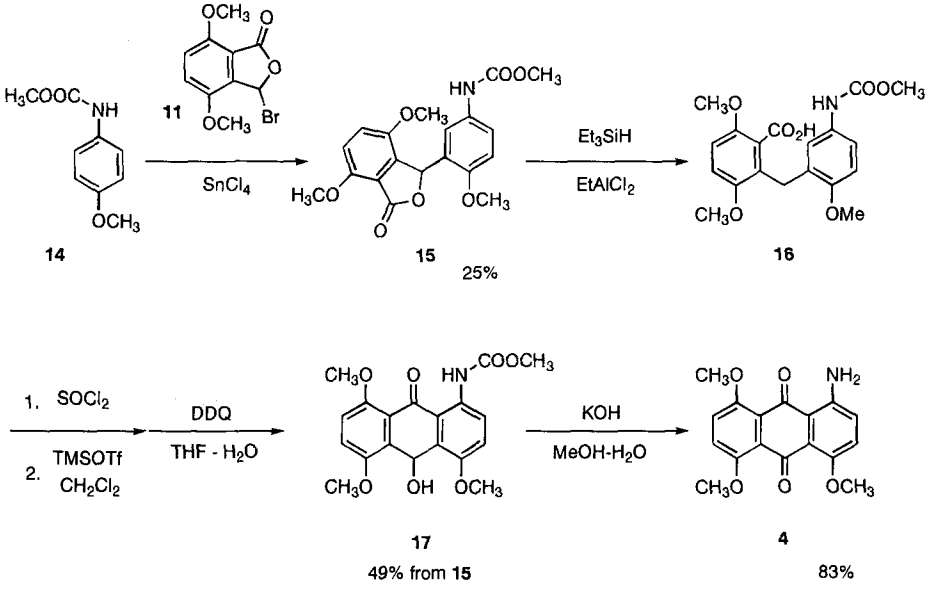

NH2
OCH3
5
Cl
O
6
Et3N / benzene
HN
7
69 %
hν
benzene
8
9
83% (1:1)
LiAlH4
THF
ClCO2CH3
pyridine
R = CO2CH3
10
73%
11
Br
SnCl4
H3CO
12a (diastereomeric mixture)
12b (diastereomeric mixture)
38% as a mixture of all isomers
Et3SiH
EtAlCl2
CO2H
13a
13b
57% as a mixture of 13a and 13b
1. SOCl2 benzene
2. TMSOTf CH2Cl2
DDQ
THF - H2O
BBr3
CH2Cl2
2
21%
1
89%

Dynemicins A, L, M, N and Q were isolated from the broth of *Micromonospora chersina* M956-1 strain.[2] Dynemicin H was synthesized by the reductive rearrangement of dynemicin A.[6]

Pentacyclic quinones **1** and **2** were synthesized as shown in **Scheme 1**. Acylation of *p*-anisidine (**5**) with cyclohex-1-enecarbonyl chloride (**6**) afforded the α, β-unsaturated amide **7**. Photochemical cyclization of **7** by high-pressure mercury lamp irradiation proceeded smoothly to afford the *cis*-**8** and the *trans*-**9** with no diastereoselectivity.[7] After separation of diastereomers by silica gel column chromatography, *cis*-**8** was subjected to lithium aluminum hydride reduction followed by protection with methylchloroformate to give the carbamate **10**. Friedel-Crafts alkylation of **10** with 3-bromo-4,7-dimethoxyphthalide **11** gave an inseparable mixture **12** of four isomers. Reductive cleavage of the lactone **12** by Et_3SiH-$MeAlCl_2$ gave a carboxylic acid mixture **13**. Intramolecular Friedel-Crafts acylation of **13** gave unstable anthracenols, that were immediately subjected to dichlorodicyanoquinone (DDQ) oxidation to give a pentacyclic quinone **2**. Demethylation of **2** was performed by BBr_3 treatment to give the trihydroxy quinone **1**. The *trans*-**9** was likewise transformed to the pentacyclic quinone **2**.

Trihydroxyaminoanthraquinone **3** was prepared according to the reported procedure.[8] Since *O*-methylation of **3** was unsuccessful, trimethoxyaminoanthraquinone **4** was synthesized as follows (**Scheme 2**). Friedel-Crafts alkylation of **14** with **11** gave **15** as a sole product. Reductive lactone cleavage and intramolecular Friedel-Crafts acylation, followed by DDQ oxidation, gave the ketol **17**. Deprotection of **17** and further oxidation to the quinone **4** were simultaneously done in one pot by refluxing with sodium hydroxide in methanol under air.

Scheme 2



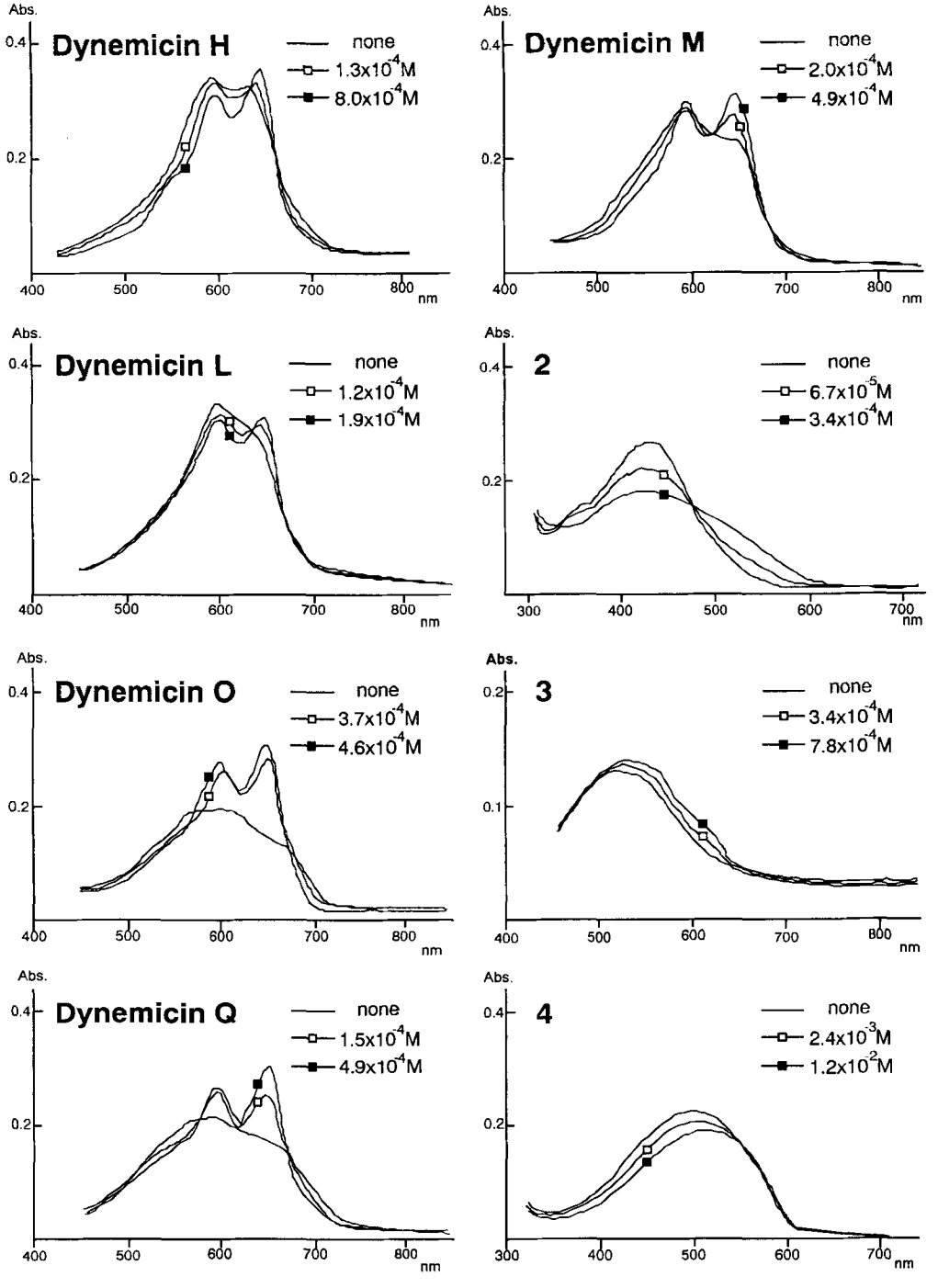


Figure 2. Effect of DNA on the visible absorption spectra of dynemicins and *aza*-anthraquinones
Calf thymus DNA (concentration indicated) was added to a solution of a drug (30 μM) in 2% DMSO, 1 mM sodium phosphate (pH 7.3)-1 mM EDTA.

Interaction of DNA with the non-diynene dynemicins and the synthesized *aza*-anthraquinones was examined.

Addition of excess calf thymus DNA to a drug solution (2% DMSO, 1 mM pH 7.3 sodium phosphate, 1 mM EDTA) induced a red shift in the UV absorption spectrum of the drug (**Figure 2**).[9] Scatchard analysis of the UV absorption shifts gave apparent association constants of 1.0 x 10^4 M^{-1} to 2.1 x 10^4 M^{-1} for dynemicins H, L, M, O and Q, as indicated in **Figure 3.** All dynemicins examined showed similar binding ability with DNA.

As expected, binding ability of **1** - **4** with DNA varied depending on their structures. The determined association constant of **3** was 9 times greater than that of **4**. Compound **2** had a large association constant with a clear isosbestic point in the UV absorption spectrum, but the association constant of the demethylated product **1** could not be obtained since no isosbestic point was observed.

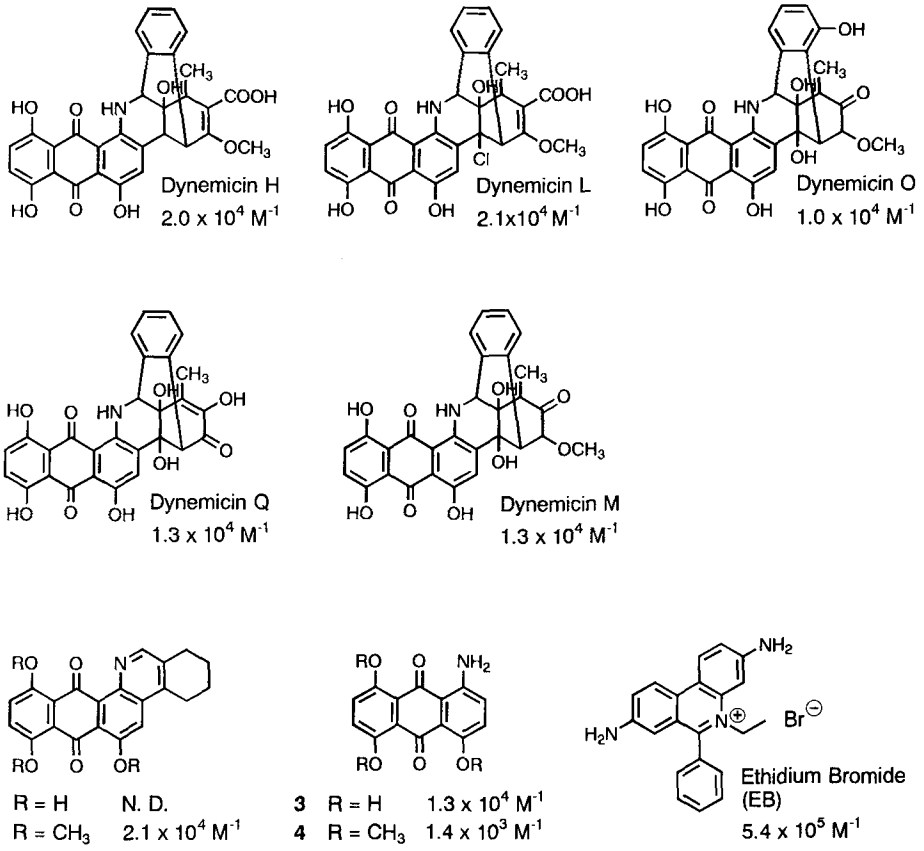


Figure 3. Association constants of non-diynene dynemicins and *aza*-anthraquinones

Since supercoiled DNA incubated with these non-diynene dynemicins was retarded on electrophoresis (**Figure 4**), intercalative binding of these drugs to DNA is suggested. On the other hand, the synthetic anthraquinones **1-4** had no effect on electrophoretic mobility, showing no evidence of their intercalative binding. Though the binding mode of **1-4** to DNA remains unknown, the fact suggested that the appendage attached to the anthraquinone, present in dynemicins, might be playing important roles for intercalation.

Currently, synthesis of anthraquinones more closely related to the dynemicin substructure is in progress, for further investigation of the function of the *aza*-anthraquinone moiety of dynemicins.

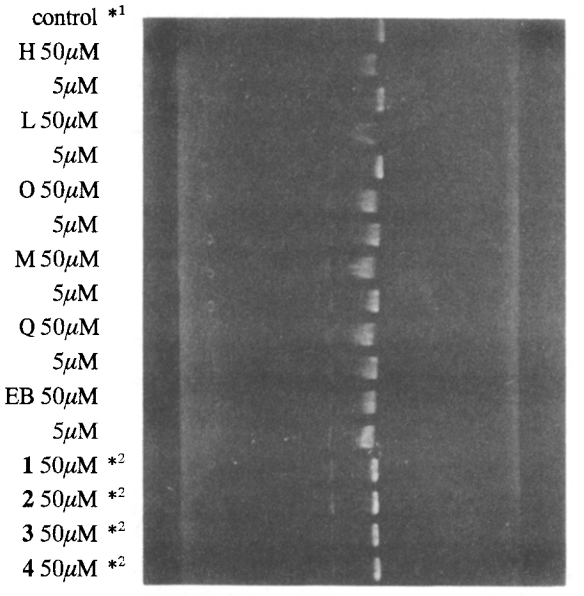


Figure 4. Effect of dynemicins and *aza*-anthraquinones on electrophoretic migration of supercoiled plasmid DNA

***1** Supercoiled plasmid DNA (pBR322) only.
***2** No shift was observed at 100-fold excess of the drugs.

ACKNOWLEDGEMENT

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9. All sets of UV spectra were obtained unequivocally at 6 to 20 different DNA concentrations per compound. Spectra at representative DNA concentrations were shown in **Figure 2**.

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