A solution of **7a** (1.1 g, 1.5 mmol) in a mixture of EtOH (60 mL) and H_2O (50 mL) was stirred with $Ba(OH)_2 \cdot 8H_2O$ (0.553 g, 1.75 mmol) for 20 h at 25 °C. A solution of NH_4HCO_3 (0.152 g, 1.92 mmol) in H_2O (3 mL) was then added and the precipitated $BaCO_3$ was filtered off. The filtrate was partially evaporated, acidified to pH 5.0 with AcOH, and freeze-dried. TLC (cellulose, pH 7.4 phosphate buffer) showed a UV-absorbing spot at R_f 0.21 and a blue fluorescent spot at R_f 0.64. The solid was dissolved in 3% NH_4HCO_3 (15 mL); the pH was adjusted to 9.7 with ammonia; and the solution was applied onto a DEAE-cellulose column (Whatman DE52, HCO_3^- form, 100 g). The column was eluted first with distilled H_2O (150 mL) to remove inorganic salts and then with 3% NH_4HCO_3. The first 90 mL of 3% NH_4HCO_3 eluate (R_f 0.64 spot) were combined and freeze-dried to obtain **9** as a pale-yellow powder (0.654 g). Further elution of the column with 350 mL of 3% NH_4HCO_3 and freeze-drying gave **3a** as a bright yellow powder (0.128 g).

Aminopterin γ-Amides via Sodium Hydroxide Hydrolysis (Method F). Aminopterin γ-(1-Adamantylamide) (3b). A solution of **7b** (1.05 g, 1.46 mmol) in a mixture of MeOH (40 mL) and 0.25 N NaOH (60 mL) was kept at 25 °C for 72 h, then acidified to pH 4.5 with 10% AcOH, refrigerated for 1 h, and filtered to give, after drying in vacuo, a bright yellow powder (0.714 g).

Other aminopterin γ-amides (**3a**, **3c–g**) and the methotrexate γ-amide **3i** were prepared by the same method as the amide **3b**, with minor differences in the percent of MeOH (40–60%) and in the length of reaction (14–28 h); yields and melting points are given in Table IV.

Aminopterin γ-(3,4-Dihydroxyanilide) (3h) (Method G). **3g** (100 mg, 0.168 mmol) was added to 4 mL of 0.5 M boron tris(trifluoroacetate) in TFA at 0 °C. After 15 min, the solution was allowed to warm up to 25 °C, at which temperature it was kept for 2 h. The solvent was removed in vacuo, MeOH added, and the solution reevaporated. Another portion of MeOH was added, and the solution was concentrated to a volume of 3 mL and added dropwise to stirred Et_2O (40 mL). The precipitated solid was filtered and dried at 60 °C in vacuo over P_2O_5 with protection from light; yield 101 mg.

Similar treatment of the methotrexate derivative **3i** afforded **3j** (cf. Table IV).

Methotrexate α-Benzyl Ester γ-(3,4-Methylenedioxyanilide) (7h) (Method H). **10** (2.78 g, 7.9 mmol) was added in small portions to a stirred solution of diethyl phosphorocyanidate (3.87 g, 2.37 mmol) and Et_3N (2.4 g, 23.7 mmol) in dry DMF (250 mL). Complete solution was achieved within 30 min. After 4 h, **5g** (4.17 g, 7.9 mmol) and Et_3N (1.6 g, 1.58 mmol) were added, and stirring was continued for 24 h at 25 °C. The solvent was removed in vacuo, and the residue was suspended in $CHCl_3$ (450 mL). The resulting mixture was washed by stirring with 10% NH_4OH (400 mL). The solid and the organic phase were combined, and the $CHCl_3$ was evaporated. Flash chromatography on silica gel with 5:5:1 $CHCl_3$–MeCN–MeOH as the eluent was performed, and appropriate fractions containing pure **7h** were pooled and evaporated. The residue was taken up in a small volume of MeOH, and the solution was added in a slow stream to stirred Et_2O. The precipitated solid was filtered and dried in vacuo at 100 °C over P_2O_5; yield 4.63 g.

Acknowledgment. This work was supported in part by Grants CA25394 (A.R.), CA19589 (A.R., M.M.W.), and CA41461 (J.H.F.) from the National Cancer Institute, DHHS. The technical assistance of Paul Genereux in obtaining in vivo antitumor data and of Judson Ratliff in obtaining some of the L1210 cell culture data is gratefully acknowledged.

Registry No. **3a**, 102650-87-3; **3b**, 102650-88-4; **3c**, 102650-89-5; **3d**, 102650-90-8; **3e**, 102650-91-9; **3f**, 102650-92-0; **3g**, 102650-93-1; **3h**, 102682-15-5; **3i**, 102650-94-2; **3j**, 102650-95-3; **4a**, 102651-05-8; **4b**, 102651-06-9; **4c**, 29421-22-5; **4d**, 102651-07-0; **4e**, 102651-08-1; **4f**, 102651-09-2; **4g**, 102651-10-5; **5a**·TsOH, 102651-12-7; **5b**·HCl, 102651-13-8; **5d**·TsOH, 102651-15-0; **5e**·TsOH, 102651-17-2; **5f**·TsOH, 102651-19-4; **5g**·TsOH, 102651-21-8; **6**, 89043-75-4; **7a**, 102650-96-4; **7b**, 102650-97-5; **7c**, 102650-98-6; **7d**, 102650-99-7; **7e**, 102651-00-3; **7f**, 102651-01-4; **7g**, 102651-02-5; **7h**, 102651-03-6; **8**, 95485-01-1; **9**, 102651-04-7; **10**, 19741-14-1; DHFR, 9002-03-3; *t*-$BuNH_2$, 75-64-9; $PhCH_2NH_2$, 100-46-9; 3,4-$Cl_2C_6H_3CH_2NH_2$, 102-49-8; 2,6-$Cl_2C_6H_3CH_2NH_2$, 6575-27-5; $PhNH_2$, 62-53-3; 3,4-$(OCH_2O)C_6H_3NH_2$, 14268-66-7; 3,4-$(OH)_2C_6H_3NH_2$, 13047-04-6; L-BOCGluOCH$_2$Ph, 30924-93-7; TsOH, 104-15-4; 1-adamantylamine, 768-94-5; methotrexate, 59-05-2.

Synthesis of Anthraquinonyl Glucosaminosides and Studies on the Influence of Aglycone Hydroxyl Substitution on Superoxide Generation, DNA Binding, and Antimicrobial Properties

Hanley N. Abramson,*† Jon W. Banning,† Joseph P. Nachtman,† Edward T. Roginski,† Maniklal Sardessai,† Henry C. Wormser,† Jender Wu,† Ziad Nagia,† Ronald R. Schroeder,‡ and Maria Margarida Bernardo‡

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, and Department of Chemistry, Wayne State University, Detroit, Michigan 48202. *Received July 25, 1985*

A series of anthraquinonyl glucosaminosides (**10a–e**) were synthesized by Koenigs–Knorr glycosidation of the corresponding aglycones (**11a–e**) with bromo sugar **12** followed by saponification. These glycosides were intended to serve as models to study the role played by the hydroxyl substituents on the aglycone portion of the antitumor anthracycline antibiotics. Superoxide generation as measured in rat heart sarcosomes was found to increase with the addition of successive hydroxyl groups to the anthraquinone nucleus. The 1,8-dihydroxy pattern was determined to generate significantly less superoxide than the 1,4-dihydroxy pattern. Hydroxyl substitution was also observed to stabilize the complex formed between the anthraquinones and DNA and was required for antibacterial activity against a number of Gram-positive organisms.

The anthracycline antibiotics daunomycin (**1**) and adriamycin (**2**) have achieved prominence in recent years due to their demonstrated clinical activity against a wide variety of tumor types.[1,2] Their utility, however, is severely limited by a tendency to produce potentially fatal dose-dependent cardiotoxicity.[2] Although the mechanism of action of these drugs has yet to be determined, there is substantial evidence that, with certain notable exceptions

†Department of Pharmaceutical Sciences.
‡Department of Chemistry.

(1) Blum, R. H.; Carter, S. K. *Ann. Intern. Med.* **1974**, *80*, 249.
(2) Carter, S. K. *J. Natl. Cancer Inst.* **1975**, 55, 1265.

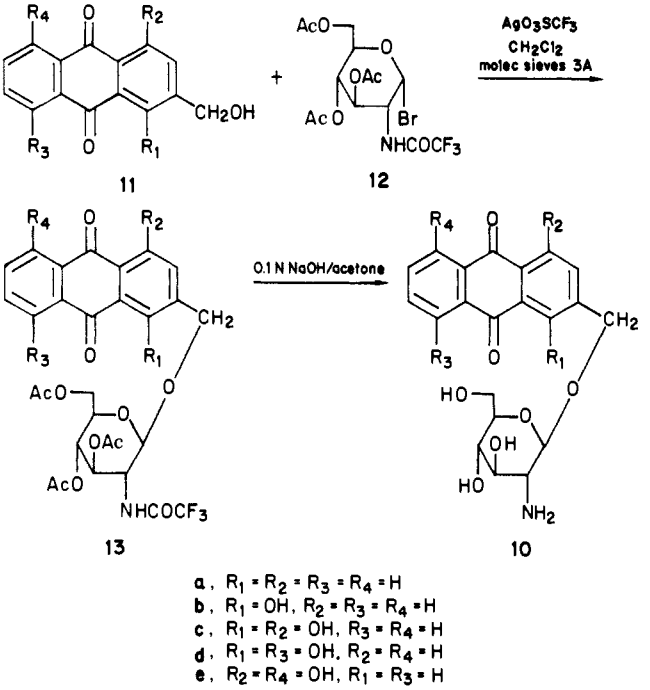
0022-2623/86/1829-1709$01.50/0

[e.g., *N*-(trifluoroacetyl)adriamycin 14-valerate],[3-5] antineoplastic activity correlates with intercalative binding to double-helical DNA.[6-8] In addition there is a growing body of evidence indicating that the biological actions of the anthracyclines can be attributed to a variety of effects that they are known to exert on cell membranes.[9-14]

The cardiotoxicity of the anthracyclines has been related to the redox cycling of an intermediate semiquinone in vivo giving rise to reactive oxygen species such as superoxide anion and hydroxyl radical, which in turn produce lipid peroxidation and DNA lesions.[15-23] The relative deficiency of the protective enzymes superoxide dismutase, glutathione peroxidase, and catalase in cardiac tissue renders the heart particularly sensitive to the destructive effects of these radicals.[24-27]

Modifications in the substituents on the B and D rings are known to specifically influence the biological properties of the anthracyclines. For example, 4-demethoxydaunomycin (**3**), which exhibits oral activity in contrast to daunomycin and adriamycin, is 4–8 times more potent than either of these drugs.[28] Carminomycin (**4**) has also demonstrated favorable antitumor activity in clinical trials.[29,30] However, both **3**[31] and **4**[32] are reported to be

Scheme I

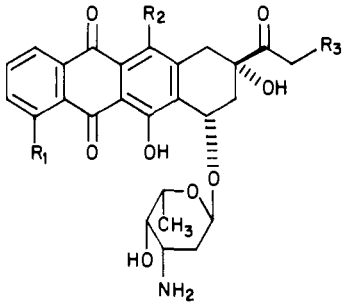


3–5 times as cardiotoxic as adriamycin. Several 11-deoxyanthracyclines including 11-deoxydaunomycin (**5**)[33] and 11-deoxyadriamycin (**6**)[34,35], as well as their 4-demethoxy derivatives **7** and **8**,[34] and aclacinomycin A (**9**)[36] have significant antitumor activity and reduced cardiotoxicity compared with **1** and **2**.

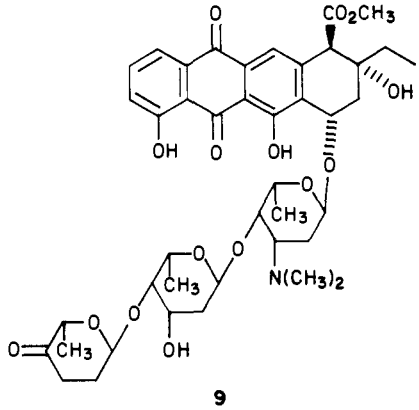
Thus, the B, C, and D rings (equivalent to the anthraquinone nucleus) and the oxygenated functionalities contained therein may be viewed as playing key roles in determining the biological properties of the anthracyclines. Among other things, this portion of the molecule is responsible for intercalation into DNA[6-8] and for the redox characteristics[15-23] associated with this class of antitumor agents. In an effort to assess the relative contributions played by the oxygen functionalities on the B and D rings we have prepared a series of five anthraquinonyl glucosaminosides (**10a–e**) for use as water-soluble model compounds in a pilot study of the effect of hydroxyl substitution on certain biological properties that are characteristic of the anthracyclines. Cost factors dictated the choice of glucosaminyl sugars in preference to their daunosaminyl counterparts, although the binding of the glucosaminyl analogues of **1** and **2** to DNA is known to be much weaker than that of the parent antibiotics.[37,38]

Chemistry. The aglycone **11a** was obtained from 2-bromomethylanthraquinone by acetate displacement followed by hydrolysis as described by Etienne and Camier.[39]

(3) Israel, M.; Modest, E. J.; Frei, E., III *Cancer Res.* **1975**, *35*, 1365.
(4) Sengupta, S. K.; Seshadri, R.; Modest, E. J.; Israel, M. *Proc. Am. Assoc. Cancer Res.* **1976**, *17*, 109.
(5) Chuang, L. F.; Kawahata, R. T.; Chuang, R. Y. *FEBS Lett.* **1980**, *117*, 247.
(6) Zunino, F.; Gambetta, R. A.; DiMarco, A.; Zaccara, A. *Biochim. Biophys. Acta* **1972**, *277*, 489.
(7) Plumbridge, T. W.; Brown, J. R. *Biochim. Biophys. Acta* **1977**, *479*, 441.
(8) Moliner-Jurnel, C.; Malfoy, B.; Reynaud, J. B.; Aubel-Sadron, G. *Biochem. Biophys. Res. Com.* **1978**, *84*, 441.
(9) Skovsgaard, T. *Biochem. Pharmacol.* **1977**, *26*, 215.
(10) Bowen, D.; Goldman, I. D. *Cancer Res.* **1975**, *35*, 3054.
(11) Zwelling, L. A.; Michaels, S.; Erickson, L. C.; Ungeleider, R. S.; Nichols, M.; Kohn, K. W. *Biochemistry* **1981**, *20*, 6553.
(12) Murphee, S. A.; Cunningham, L. S.; Hwang, K. M.; Sartorelli, A. C. *Biochem. Pharmacol.* **1976**, *25*, 1227.
(13) Tritton, T. R.; Murphee, S. A.; Sartorelli, A. C. *Biochem. Biophys. Res. Com.* **1978**, *84*, 802.
(14) Wingard, L. B., Jr.; Tritton, T. R.; Egler, K. A. *Cancer Res.* **1985**, *45*, 3529.
(15) Sato, S.; Iwaizumi, M.; Handa, K.; Tamura, Y. *Gann* **1977**, *68*, 603.
(16) Handa, K.; Sato, S. *Gann* **1976**, *67*, 523.
(17) Goodman, J.; Hochstein, P. *Biochem. Biophys. Res. Commun.* **1977**, *77*, 797.
(18) Bachur, N. R.; Gordon, S. L.; Gee, M. V. *Mol. Pharmacol.* **1977**, *13*, 901.
(19) Bachur, N. R.; Gordon, S. L.; Gee, M. V. *Cancer Res.* **1978**, *38*, 1745.
(20) Bachur, N. R.; Gordon, S. L.; Gee, M. V.; Kon, H. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 954.
(21) Doroshow, J. H.; Reeves, J. *Proc. Am. Assoc. Cancer Res.* **1980**, *21*, 266.
(22) Doroshow, J. H.; Davies, K. J. A. *Biochem. Pharmacol.* **1983**, *32*, 2935.
(23) Doroshow, J. H. *Cancer Res.* **1983**, *43*, 460.
(24) Thayer, W. S. *Chem.-Biol. Interact.* **1977**, *19*, 265.
(25) Hien, P. V.; Kovacs, K.; Mathovics, B. *Enzyme* **1975**, *19*, 1.
(26) Doroshow, J. H.; Locker, D. Y.; Myers, C. E. *J. Clin. Invest.* **1980**, *65*, 128.
(27) Revis, N. W.; Marusic, N. *J. Mol. Cell. Cardiol.* **1978**, *10*, 945.
(28) DiMarco, A.; Casazza, A. M.; Pratesi, G. *Cancer Treat. Rep.* **1977**, *61*, 893.
(29) Perevodchikova, N. I.; Lichinister, M. R.; Gorbunova, V. A. *Cancer Treat. Rep.* **1977**, *61*, 1705.
(30) Baker, L. H.; Kessel, D. H.; Comis, R. L.; Reich, S. D.; DeFuria, M. D.; Crooke, S. T. *Cancer Treat. Rep.* **1979**, *63*, 899.
(31) Casazza, A. M. *Cancer Treat. Rep.* **1979**, *63*, 835.
(32) Kirchner, E.; Guttner, J.; Fritsch, R. S.; Hartly, A.; Hoffman, H. *Acta Pharmacol. Toxicol.* **1981**, *48*, 87.
(33) Cassinelli, G.; DiMatteo, F.; Forenza, S.; Ripamonti, M. C.; Rivola, G.; Arcamone, F.; DiMarco, A.; Casazza, A. M.; Saranzo, C.; Pratesi, G. *J. Antibiot.* **1980**, *33*, 1468.
(34) Umezawa, H.; Takahashi, Y.; Kinoshita, M.; Naganawa, H.; Tatsuta, K.; Takeuchi, T. *J. Antibiot.* **1980**, *33*, 1581.
(35) Arcamone, F.; Cassinelli, G.; DiMatteo, F.; Forenza, S.; Ripamonti, M. C.; Giovanni, R.; Vigeva, A.; Clardy, J.; McCabe, T. *J. Am. Chem. Soc.* **1980**, *102*, 1462.
(36) Oki, T. In *Anthracyclines: Current Status and New Developments*; Crooke, S. T., Reich, S. D., Eds.; Academic: New York, 1980; pp 323–342.
(37) Penco, S. *Chim. Ind. (Milan)* **1968**, *50*, 908.
(38) Israel, M.; Murray, R. J. *J. Med. Chem.* **1982**, *25*, 24.



1, $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$
2, $R_1 = OCH_3$, $R_2 = R_3 = OH$
3, $R_1 = R_3 = H$, $R_2 = OH$
4, $R_1 = R_2 = OH$, $R_3 = H$
5, $R_1 = OCH_3$, $R_2 = R_3 = H$
6, $R_1 = OCH_3$, $R_2 = H$, $R_3 = OH$
7, $R_1 = R_2 = R_3 = H$
8, $R_1 = R_2 = H$, $R_3 = OH$



9

Compounds **11b–d** were synthesized by the reductive hydroxymethylation procedure of Bredereck et al.[40] from 1-hydroxy-, 1,4-dihydroxy-, and 1,8-dihydroxyanthraquinones, respectively. Aloe emodin, **11e**, was prepared by ferric chloride oxidation of commercial aloin.[41]

Koenigs–Knorr glycosidations (Scheme I) of **11a–e** with bromo sugar **12**[42] were conducted in methylene chloride in the presence of silver trifluoromethanesulfonate and molecular sieves. In each case the product, obtained in yields of 21–63% after purification on column chromatography, was shown to be the β-glycoside (**13a–e**) as determined by examination of the 300-MHz ^1H NMR spectra. The doublet that appeared in each of the spectra in the region 4.79–4.86 ppm was assigned to the anomeric proton and this was confirmed by decoupling experiments. The coupling constant $J_{1',2'}$ varied from 8.3 to 8.6 Hz, which is consistent with the β assignment;[43] the α-anomer would be expected to have a coupling constant of approximately 4 Hz.[43] This assignment is in harmony with the general preference for the formation of 1,2-*trans*-glycosides in the Koenigs–Knorr reaction[44] and the fact that bromo sugar **12** reacts with other alcohols to give almost exclusively the β-isomer.[37,38,45] The NMR spectra of **13a–e** also revealed the existence of hindered rotation about the glycoside bond as evidenced by two distinct doublets (one at 4.73–4.83 ppm and the other at 4.98–5.06 ppm, the geminal coupling constant being in the range of 13–15 Hz) attributed to the methylene group attached to the anthraquinone ring.

Mild base hydrolyses of the protected glycosides **13a–e** were performed in a mixture of acetone and 0.1 N NaOH to provide the desired compounds **10a–e** as amorphous solids. With the exception of **10b**, attempts to prepare the hydrochloride salts of the amino sugars led to decomposition upon purification.

Table I. Effect of Glycosides on Superoxide Production in Rat Heart Sarcosomes[a] and Comparison with Half-Wave Potentials

compd	nmol acetylated cytochrome *c* reduced/min per mg protein (mean ± SEM, $N = 3$)	$E_{1/2}$, V
control	1.34 ± 0.20[b]	
1	2.76 ± 0.57	−0.77
10a	2.31 ± 0.43	−0.97
10b	5.49 ± 0.20	−0.73
10c	12.80 ± 0.34[c]	−0.72
10d	10.50 ± 0.63	−0.68
10e	10.10 ± 0.54	−0.70

[a] Data obtained with sarcosomes prepared concurrently from six rats and equimolar concentrations (0.1 mM) of each compound. [b] Rate of reduction with no test compound present. [c] Significantly different from **10d** and **10e** ($p < 0.05$).

Table II. Binding of Glycosides to DNA

compd	K_d,[a] μM	n	ΔT_m, °C
1	1.28	0.20	16.0
10a		0	1.4
10b	4.30	0.33	5.6
10c	2.98	0.25	8.5
10d	2.07	0.30	8.2
10e	3.00	0.22	7.7

[a] Inverse of K_a; see the Experimental Section.

Results and Discussion

The ability of each of the glycosides **10a–e** to stimulate superoxide radical production in rat heart sarcosomes was determined and the results are presented in Table I. The addition of each successive hydroxyl group to the anthraquinone ring approximately doubles the amount of superoxide produced as determined by the rate of reduction of superoxide dismutase-inhibitable acetylated cytochrome *c*. It is interesting to note that the generation of superoxide by the two 1,8-dihydroxylated anthraquinones **10d** and **10e** is significantly less (as determined using one-way analysis of variance) than that generated by the 1,4-dihydroxylated compound **10c**, an observation that may have relevance in explaining the reduced cardiotoxicity of 11-deoxyanthracyclines such as **9**[36] relative to **1** and **2**.

Compounds **10b–e** upon interaction with calf thymus DNA each produced bathochromic and hypochromic shifts in the visible region of the spectrum with well-defined isosbestic points. They also increased the melting temperature (T_m) of the DNA (Table II). The nonhydroxylated anthraquinone **10a** did not bind to DNA and only slightly increased the T_m value. Henry[46] has similarly reported that the daunosaminyl analogue of **10a** binds very weakly to DNA with a ΔT_m of 2.2 °C. The monohydroxylated anthraquinone (**10b**) was observed to bind to DNA much better than **10a** but not as firmly as the three dihydroxylated compounds (**10c–e**). Thus, it appears that OH substitution on the anthraquinone ring provides stabilization of the complex. This result is in agreement with the findings of Bennett et al.[47] who studied the

(39) Etienne, A.; Camier, J. *Compt. Rend.* **1960**, *251*, 100.
(40) Bredereck, K.; Metwally, S. A.; Koch, E.; Weckmann, R. *Liebigs Ann. Chem.* **1975**, 972.
(41) Hay, J. E.; Haynes, L. J. *J. Chem. Soc.* **1956**, 3141.
(42) Wolfrom, M. L.; Bhat, H. B. *J. Org. Chem.* **1967**, *32*, 1821.
(43) Horton, D. *J. Org. Chem.* **1964**, *29*, 1776.
(44) Wulff, G.; Rohle, G. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157.
(45) Strachan, R. G.; Ruyle, W. V.; Shen, T. Y.; Hirschmann, R. *J. Org. Chem.* **1966**, *31*, 507.
(46) Henry, D. W. In *Cancer Chemotherapy*, Sartorelli, A. C., Ed.; ACS Symposium Series No. 30, American Chemical Society: Washington, DC, 1976; p 41.

Table III. Antibacterial Activity of Glycosides[a]

	MIC values, μg/mL							
compd	*S. aureus* ATCC 25923	*S. aureus* ATCC 9144	*S. aureus*[b] WSU-196E	*Staph. epidermitis* ATCC 12228	*Strep. pyogenes* ATCC 19615	*B. cereus*[b] WSU-60	*B. subtilis* ATCC E6051	*C. diphtheriae*[b] WSU-280
1	20	<5	<5	<5	<5	<5	<5	<5
2	200	20	10	<5	<5	10	20	20
10a	>200	>200	>200	200	100	>200	>200	>200
10b	200	200	200	20	20	100	50	100
10c	100	50	>200	20	10	50	50	100
10d	100	200	200	50	50	50	50	50
10e	>200	50	>200	20	10	200	>200	100

[a] The minimum inhibitory concentrations (MICs) were determined by the agar dilution method of Mitscher et al.[51] Compounds 1 and 2 were dissolved in water, and compounds 10a–e were dissolved in dimethyl sulfoxide and the resultant mixtures added to the agar prior to setting. [b] Obtained from the Department of Family and Consumer Resources, Wayne State University, Detroit, MI.

DNA-binding ability of a series of hydroxylated and nonhydroxylated 2-substituted anthraquinones. It is also consistent with the observations of others[48-50] that methylation of the C-6 and C-11 hydroxyl groups in anthracyclines leads to reduced DNA binding and loss of antitumor activity. Quadrifoglio et al.[50] attributed this reduction in DNA binding more to steric effects than to an alteration in charge distribution in the chromophore. Our results would appear to indicate that electronic factors probably play an important role in the binding of the planar chromophore. However, the stereochemistry of the attachment of the sugar to the chromophore has also been shown to be an important factor in determining the extent of DNA binding. Henry[46] has found that in a pair of diastereomers of daunosaminyl glycosides of tetrahydronaphthacene quinone analogues of daunomycin (lacking substituents at C-9 as well as the methoxy group at C-4) the isomer with the natural *S* configuration at C-7 (ΔT_m = 12.6 °C) bound much more firmly to DNA than did the isomer with the unnatural *R* configuration (ΔT_m = 6.8 °C). The latter value is in the general range found for **10c–e**. Further work is required to determine if these glycosides form intercalation complexes with DNA.

In a survey of the antibacterial activity of the glycosides against Gram-positive organisms (Table III) it is apparent that hydroxylation of the anthraquinone nucleus is required for substantial inhibitory activity. While no clear relationship exists between the substitution pattern and antibacterial activity, the 1,4-dihydroxy substituted compound (**10c**) appears to be superior to the 1-hydroxy compound (**10b**) against four of the organisms.

Experimental Section

All organic starting materials and reagents were obtained from Aldrich Chemical Co. except for aloin (Pfaltz and Bauer). Solvents used were reagent grade and obtained from Fisher Scientific Co. Methylene chloride was distilled over phosphorus pentoxide prior to use.

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-33 infrared spectrometer. Proton magnetic resonance spectra were obtained with a Nicolet QE-300 spectrometer and are reported as parts per million (ppm) relative to tetramethylsilane. Ultraviolet and visible spectra were recorded on a Beckman Model 35 spectrometer. Mass spectra were determined with a Kratos MS80RFA mass spectrometer by direct chemical ionization using isobutane as the reagent gas. Electrochemical data on the glycosides were obtained by cyclic voltammetry in anhydrous dimethylformamide solution containing 0.1 M tetraethylammonium perchlorate as described by Lintvedt et al.[52] Combustion analyses were performed by Midwest Microanalytical Laboratory, Indianapolis, IN.

Analytical thin-layer chromatography was carried out on prelayered silica gel GF, 0.25-mm plates (Analtech), visualizing with either UV light or ceric sulfate–sulfuric acid charring. Solvent systems used included (A) methanol–chloroform (1:49) and (B) chloroform–methanol–water (120:20:1). Column chromatography was performed with silica gel 60 (70–230 mesh; E. Merck).

Koenigs–Knorr Glycosidation of Hydroxymethylanthraquinones. 2-[[3,4,6-Tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyloxy]methyl]-9,10-dihydro-9,10-anthracenedione (13a). A mixture of 0.50 g (2.10 mmol) of 2-hydroxymethylanthraquinone (**11a**), 1.09 g (2.35 mmol) of 3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-α-D-glucopyranosyl bromide (**12**),[42] 0.59 g (2.31 mmol) of silver trifluoromethanesulfonate, 0.50 g of molecular sieves type 3A, and 50 mL of dry CH_2Cl_2 was stirred at room temperature under nitrogen in the dark for 4 h. The course of the reaction was monitored by TLC (A). Two elutions were required to adequately separate the glycoside product from **11a**. The reaction mixture was treated with 5% aqueous $NaHCO_3$ solution, stirred for 30 min, and filtered. The residue was washed with CH_2Cl_2 and the filtrate was concentrated to dryness. Column chromatography of the crude product afforded 165 mg of **11a** upon elution with C_6H_6–$CHCl_3$ (2:3). Subsequent elution with $CHCl_3$ yielded **13a** (0.59 g, 45%) as nearly colorless crystals: mp 273–275 °C dec; NMR ($CDCl_3$) δ 2.04 (s, 6 H, $OCOCH_3$), 2.13 (s, 3 H, $OCOCH_3$), 3.76 (ddd, 1 H, C5′H; $J_{4',5'}$ = 9.5, $J_{5',6'a}$ = 4.7, $J_{5',6'b}$ = 2.4 Hz), 4.11 (ddd, 1 H, C2′H; $J_{1',2'}$ = 8.4, $J_{2',NH}$ = 8.4, $J_{2',3'}$ = 10.5 Hz), 4.21 (dd, 1 H, C6′bH; $J_{6'a,6'b}$ = 12.4 Hz), 4.31 (dd, 1 H, C_6′aH), 4.79 (d, 1 H, C1′H), 4.80 and 5.06 (2d, 1 H each, anthraquinone-CH_2, J = 13.0 Hz), 5.16 (dd, 1 H, C4′H; $J_{3',4'}$ = 9.5 Hz), 5.28 (dd, 1 H, C3′H), 6.54 (d, 1 H, NH), 7.72–8.35 (m, 7 H, anthraquinone H's); IR (KBr) ν_{max} 3300, 1760, 1720, 1685 cm^{-1}; UV (95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 255 (12), 274 (3.7), 325 nm (1.3); mass spectrum, m/z 622 (MH^+), 562 (MH^+ – HOAc), 384 ($sugar^+$), 342 ($sugar^+$ – CH_2CO), 324 ($sugar^+$ – HOAc), 264 ($sugar^+$ – 2HOAc), 222 [MH^+ – (*O*-sugar)]. Anal. ($C_{29}H_{26}NO_{11}F_3$) C, H, N.

1-Hydroxy-2-[[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyloxy]methyl]-9,10-dihydro-9,10-anthracenedione (13b). This compound was obtained, using the above procedure from **11b** and **12**, as yellow crystals after column chromatography: 0.79 g (63%); mp 240–243 °C dec; NMR ($CDCl_3$) δ 2.05 (s, 6 H, $OCOCH_3$), 2.12 (s, 3 H, $OCOCH_3$), 3.78 (ddd, 1 H, C5′H; $J_{4',5'}$ = 9.6, $J_{5'6'a}$ = 4.6, $J_{5'6'b}$ = 2.4 Hz), 4.12 (ddd, 1 H, C2′H; $J_{1',2'}$ = 8.3, $J_{2',NH}$ = 8.8, $J_{2',3'}$ = 10.3 Hz), 4.20 (dd, 1 H, C6′bH; $J_{6'a,6'b}$ = 12.4 Hz), 4.33 (dd, 1 H, C6′aH), 4.82 (d, 1 H, C1′H), 4.83 and 5.03 (2d, 1 H each, anthraquinone-CH_2, J = 13.4 Hz), 5.17 (dd, 1 H, C4′H; $J_{3',4'}$ = 9.5 Hz), 5.28 (dd, 1 H, C3′H), 6.50 (d, 1 H, NH), 7.70–8.34 (m, 6 H, anthraquinone H's), 13.03 (s, 1 H, OH); IR (KBr) ν_{max} 3300, 1760, 1720, 1680, 1645 cm^{-1}; UV

(47) Bennett, S.; Sharples, D.; Brown, J. R. *J. Med. Chem.* **1982**, *25*, 369.
(48) Zunino, F.; DiMarco, A.; Zaccara, A. *Chem.-Biol. Interact.* **1979**, *24*, 217.
(49) Zunino, F.; Casazza, A. M.; Pratesi, G.; Formelli, F.; DiMarco, A. *Biochem. Pharmacol.* **1981**, *30*, 1856.
(50) Quadrifoglio, F.; Ciana, A.; Manzini, G.; Zaccara, A.; Zunino, F. *Int. J. Biol. Macromol.* **1982**, *4*, 413.
(51) Mitscher, L. A.; Leu, R. P.; Bathala, M. S.; Wu, W. N.; Beal, J. L. *Lloydia* **1972**, *35*, 157.
(52) Lintvedt, R. L.; Ranger, G.; Schoenfelner, B. A. *Inorg. Chem.* **1984**, *23*, 688.

(95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 258 (28), 266 (19), 405 nm (5.9); mass spectrum, m/z 638 (MH^+), 578 (MH^+ – HOAc), 384 ($sugar^+$), 342 ($sugar^+$ – CH_2CO), 324 ($sugar^+$ – HOAc), 264 ($sugar^+$ – 2HOAc), 238 [MH^+ – (*O*-sugar)]. Anal. ($C_{29}H_{26}NO_{12}F_3$) C, H, N.

1,4-Dihydroxy-2-[[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyloxy]methyl]-9,10-dihydro-9,10-anthracenedione (13c). This compound was prepared, using the above procedure from **11c** and **12**, as red crystals after column chromatography: 0.27 g (21%); mp 275–278 °C dec; NMR ($CDCl_3$) δ 2.05 (s, 6 H, $OCOCH_3$), 2.12 (s, 3 H, $OCOCH_3$), 3.78 (ddd, 1 H, C5′H; $J_{4',5'}$ = 9.6, $J_{5'6'a}$ = 4.6, $J_{5'6'b}$ = 2.4 Hz), 4.13 (ddd, 1 H, C2′H; $J_{1',2'}$ = 8.6, $J_{2',NH}$ = 8.8, $J_{2',3'}$ = 9.9 Hz), 4.20 (dd, 1 H, C6′H; $J_{6'a,6'b}$ = 12.1 Hz), 4.33 (dd, 1 H, C6′aH), 4.81 and 5.01 (2d, 1 H each, anthraquinone-CH_2, J = 15.1 Hz), 4.86 (d, 1 H, C1′H), 5.18 (dd, 1 H, C4′H; $J_{3',4'}$ = 9.5 Hz), 5.30 (dd, 1 H, C3′H), 6.51 (d, 1 H, NH), 7.36–8.38 (m, 5H, anthraquinone H's), 12.85 (s, 1 H, OH), 13.32 (s, 1 H, OH); IR (KBr) ν_{max} 3320, 1755, 1720, 1630 cm^{-1}; UV (95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 246 (13), 257 (11), 285 (3.8), 477 (5.3), 510 nm (3.0); mass spectrum, m/z 654 (MH^+), 653 (M^+), 594 (MH^+ – HOAc), 593 (M^+ – HOAc), 384 ($sugar^+$ – HOAc), 342 ($sugar^+$ – CH_2CO), 324 ($sugar^+$ – HOAc), 264 ($sugar^+$ – 2HOAc), 254 [MH^+ – (*O*-sugar)]. Anal. ($C_{29}H_{26}NO_{13}F_3$) C, H, N.

1,8-Dihydroxy-2-[[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyloxy]methyl]-9,10-dihydro-9,10-anthracenedione (13d). This compound was prepared, using the above procedure from **11d** and **12**, as orange crystals after column chromatography: 0.40 g (37%), mp 257–258 °C dec; NMR ($CDCl_3$) δ 2.06 (s, 6 H, $OCOCH_3$), 2.12 (s, 3 H, $OCOCH_3$), 3.78 (ddd, 1 H, C5′H; $J_{4',5'}$ = 9.6, $J_{5',6'a}$ = 4.6, $J_{5',6'b}$ = 2.4 Hz), 4.12 (ddd, 1 H, C2′H; $J_{1',2'}$ = 8.5, $J_{2',NH}$ = 8.8, $J_{2',3'}$ = 9.9 Hz), 4.20 (dd, 1 H, C6′bH; $J_{6'a,6'b}$ = 12.2 Hz), 4.33 (dd, 1 H, C6′aH), 4.82 (d, 1 H, C1′H), 4.82 and 5.03 (2d, 1 H each, anthraquinone-CH_2, J = 13.4 Hz), 5.18 (dd, 1 H, C4′H; $J_{3',4'}$ = 9.5 Hz), 5.28 (dd, 1 H, C3′H), 6.47 (d, 1 H, NH), 7.31–7.87 (m, 5H, anthraquinone H's), 12.01 (s, 1 H, OH), 12.46 (s, 1 H, OH); IR (KBr) ν_{max} 3320, 1760, 1720, 1680, 1630 cm^{-1}; UV (95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 270 (17), 485 nm (1.9); mass spectrum, m/z 654 (MH^+), 594 (MH^+ – HOAc), 384 ($sugar^+$), 342 ($sugar^+$ – CH_2CO), 324 ($sugar^+$ – HOAc), 264 ($sugar^+$ – 2HOAc), 254 [MH^+ – (*O*-sugar)]. Anal. ($C_{29}H_{26}NO_{13}F_3$) C, H, N.

1,8-Dihydroxy-3-[[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyloxy]methyl]-9,10-dihydro-9,10-anthracenedione (13e). The above procedure was used to prepare this compound from **11e** and **12** as orange crystals after column chromatography: 0.27 g (49%); mp 261–262 °C dec; NMR ($CDCl_3$) δ 2.06 (s, 6 H, $OCOCH_3$), 2.13 (s, 3 H, $OCOCH_3$), 3.75 (ddd, 1 H, C5′H; $J_{4',5'}$ = 9.6, $J_{5',6'a}$ = 4.5, $J_{5',6'b}$ = 2.2 Hz), 4.09 (ddd, 1 H, C2′H; $J_{1',2'}$ = 8.3, $J_{2',NH}$ = 8.7, $J_{2',3'}$ = 10.0 Hz), 4.20 (dd, 1 H, C_6′bH; $J_{6'a,6'b}$ = 12.4 Hz), 4.29 (dd, 1 H, C6′aH), 4.73 and 4.98 (2d, 1 H each, anthraquinone-CH_2, J = 13.6 Hz), 4.80 (d, 1 H, C1′H), 5.16 (dd, 1 H, C4′H; $J_{3',4'}$ = 9.5 Hz), 5.30 (dd, 1 H, C3′H), 6.50 (d, 1 H, NH), 7.31–7.86 (m, 5 H, anthraquinone H's), 12.06 (s, 1 H, OH), 12.07 (s, 1 H, OH); IR (KBr) ν_{max} 3320, 1755, 1720, 1680, 1630 cm^{-1}; UV (95% EtOH) λ_{max} ($\epsilon \times 10^{-3}$) 269 (13), 493 nm (1.9); mass spectrum, m/z 654 (MH^+), 594 (MH^+ – HOAc), 384 ($sugar^+$), 342 ($sugar^+$ – CH_2CO), 324 ($sugar^+$ – HOAc), 264 ($sugar^+$ – 2HOAc), 254 [MH^+ – (*O*-sugar)]. Anal. ($C_{29}H_{26}NO_{13}F_3$) C, H, N.

Base-Catalyzed Removal of Protective Groups from the Glycosides. 2-[(2-Deoxy-2-amino-β-D-glucosyloxy)methyl]-9,10-dihydro-9,10-anthracenedione (10a). A solution of 100 mg (0.16 mmol) of **13a** in 13 mL of acetone was treated with 20 mL of 0.1 N aqueous NaOH and stirred at room temperature under nitrogen for 4 h. The reaction mixture was cooled in an ice bath, treated dropwise with methanolic HCl to neutrality, and concentrated to dryness. The residue was extracted with acetone (4 × 100 mL). The combined acetone extracts were filtered and concentrated to dryness. The residue was suspended in 10 mL of cold acetone, filtered, and dried to give 30 mg (47%) of a light brown solid: single spot on TLC (B); mp 187–188 °C dec. Anal. ($C_{21}H_{21}NO_7$) C, H, N.

1-Hydroxy-2-[(2-deoxy-2-amino-β-D-glucosyloxy)methyl]-9,10-dihydro-9,10-anthracenedione (10b). This compound was prepared by the same procedure used to obtain **10a**. In this case the crude amino glycoside was treated with methanolic HCl to obtain the hydrochloride salt, which was recrystallized from MeOH–H_2O: 75 mg (48%); mp 220–221 °C dec; single spot on TLC (B). Anal. ($C_{21}H_{22}NO_8Cl\cdot H_2O$) C, H, N.

1,4-Dihydroxy-2-[(2-deoxy-2-amino-β-D-glucosyloxy)methyl]-9,10-dihydro-9,10-anthracenedione (10c). This compound was prepared as an amorphous powder by the same procedure used to obtain **10a**: 23 mg (62%); mp 157–159 °C dec; single spot on TLC (B). Anal. ($C_{21}H_{21}NO_9\cdot 0.75H_2O$) C, H, N.

1,8-Dihydroxy-2-[(2-deoxy-2-amino-β-D-glucosyloxy)methyl]-9,10-dihydro-9,10-anthracenedione (10d). This compound was prepared as an amorphous powder by the same procedure used to obtain **10a**: 20 mg (30%); mp 134–135 °C dec; single spot on TLC (B). Anal. ($C_{21}H_{21}NO_9\cdot H_2O$) C, H, N.

1,8-Dihydroxy-3-[(2-deoxy-2-amino-β-D-glucosyloxy)methyl]-9,10-dihydro-9,10-anthracenedione (10e). This compound was obtained as an amorphous powder by the same procedure used to prepare **10a**: 18 mg (53%); mp 171–173 °C dec; single spot on TLC (B). Anal. ($C_{21}H_{21}NO_9\cdot H_2O$) C, H, N.

Superoxide Generation Assay. Cytochrome c was acetylated according to the procedure of Azzi et al.[53] The extent of acetylation was determined according to the method of Finkelstein et al.[54] Sarcosomal preparations were obtained from male Sprague–Dawley rats (Charles River), which were maintained on Purina rat chow and tap water ad libitum in a temperature- (23 °C) and light- (12 h light:12 h dark) controlled environment. The rats were sacrificed by decapitation, and the ventricles were removed and freed of connective tissue and coagulated blood. The ventricles were homogenized in 4 vol (w/v) of 100 mM KCl, 5 mM histidine, pH 7.3, and centrifuged at 9000*g* for 30 min at 4 °C. The resulting supernatant was centrifuged at 100000*g* for 1 h at 4 °C. The microsomal pellet was resuspended by sonication in 10 vol (wet w/v) of 0.15 M K_3PO_4, 1.0 mM EDTA, pH 7.4, and centrifuged at 100000*g* for 1 h at 4 °C. Protein concentrations were determined by the method of Bradford.[55] The rate of superoxide generation in the rat heart sarcosomes at 37 °C was measured by the rate of increase in absorbance at 550 nm. The reaction was carried out in a 1.5-mL cuvette that contained acetylated cytochrome c (38–53 nmol), sarcosomal protein (300–500 μg), 100 nmol of the compound to be tested, and sufficient buffer (K_3PO_4 0.15 M, EDTA 1mM) to make 1 mL. Superoxide production was initiated by addition of NADPH (1 μmol in 50 μL). Two minutes following the addition of NADPH, superoxide dismutase (SOD) was added to the cuvette to determine the SOD-sensitive portion of the total rate of superoxide production. The results are expressed as nanomoles of SOD-inhibitable acetylated cytochrome c reduced per minute per milligram of protein.[23] A comparison of the ability of each of the compounds to generate superoxide was conducted by using the one-way analysis of variance performed on the MIDAS statistical program (Statistical Research Laboratory, The University of Michigan, Ann Arbor, MI). The null hypothesis for differences between means was rejected for $p < 0.05$ (F test).

DNA Binding Studies. Each of the glycosides **10a–e** was titrated with calf thymus DNA according to the procedure of Plumbridge and Brown.[7] The mole fraction of bound and free glycoside was determined by measuring absorbance change against DNA concentration. The data were fitted to a Scatchard plot by linear regression. The affinity constant (K_a) was taken as the slope, and n, the ratio of the concentration of bound glycoside to the concentration of DNA (glycoside binding sites per DNA base pair), was taken as the abscissa intercept.

For determining the DNA melting temperature (T_m), calf thymus DNA was dissolved in 3 mM Tris buffer containing 18 mM NaCl (pH 7). Glycoside and DNA concentrations were 15 μM and 150 μM, respectively. These solutions were placed in 3-mL sealed quartz cuvettes, and the absorbance change at 260 nm was plotted against cuvette temperature. The T_m was obtained by taking the midpoint of the curve between the high- and low-temperature constant-absorbance regions. The melting temperature of uncomplexed DNA under these conditions ranged from

(53) Azzi, A.; Montecucco, R.; Richter, R. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 597.
(54) Finkelstein, W.; Rosen, G. M.; Pathon, S. E.; Cohen, M. S.; Rauckman, E. J. *Biochem. Biophys. Res. Commun.* **1981**, *102*, 1008.
(55) Bradford, M. M. *Anal. Biochem.* **1976**, 72, 248.

70.0 to 70.5 °C in five different runs.

Acknowledgment. We are indebted to Drs. Robin Hood and Ole Mols and Maya Kemff of the Wayne State University Department of Chemistry for their expert assistance in obtaining NMR and MS data. This work was supported by National Cancer Institute Grant CA 32038 and Wayne State University Grant-in-Aid 167-1006.

Registry No. **10a**, 102830-98-8; **10b**, 102830-99-9; **10b**·HCl, 102831-00-5; **10c**, 102831-01-6; **10d**, 102831-02-7; **10e**, 102831-03-8; **11a**, 17241-59-7; **11b**, 24094-45-9; **11c**, 22296-59-9; **11d**, 56670-70-3; **11e**, 481-72-1; **12**, 6736-63-6; **13a**, 102830-93-3; **13b**, 102830-94-4; **13c**, 102830-95-5; **13d**, 102830-96-6; **13e**, 102830-97-7.

Thiol Addition to Quinones: Model Reactions for the Inactivation of Thymidylate Synthase by 5-*p*-Benzoquinonyl-2′-deoxyuridine 5′-Phosphate

Madhavi K. Vadnere, Linda Maggiora, and Mathias P. Mertes*

Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045. *Received December 9, 1985*

The reaction of methyl mercaptoacetate (**5**) with phenyl-*p*-benzoquinone (**6**) or 5-*p*-benzoquinonyl-3′,5′-di-*O*-acetyl-2′-deoxyuridine (**10**) resulted in the formation of the three possible adducts to the quinone rings of **6** and **10**; an additional product in the reaction with **10** was the unsubstituted hydroquinone (**14**). Both reactions were found to be solvent dependent; in buffered aqueous acetonitrile the meta and para adducts of **10** were formed in the ratio of 2:1. In ethyl acetate the ortho adduct and the reduction product of **10** were isolated in a ratio of 2:3. The second-order rate constant for the reaction of **5** with **10** in acetonitrile was 0.53 M^{-1} s^{-1}; the reaction was accelerated by the addition of water. Although the initially proposed mechanism-based enzyme inactivation cannot be excluded, the results of the model reactions support the alternative mechanism, active-site thiol addition to the quinone ring. If this is true the title compound would be classed as an affinity label, not a mechanism-based inhibitor.

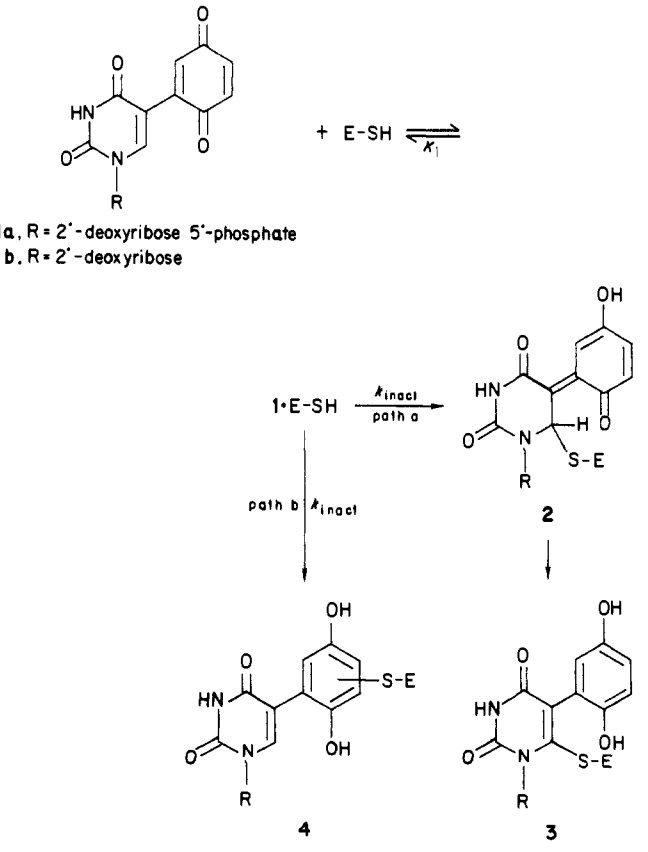
The design and synthesis of affinity labels as enzyme inhibitors has been a useful approach for probing the mechanism of enzyme catalysis and for the development of agents for disease control. Three criteria normally are employed in the verification that a compound is an affinity label:[1] (1) A reversible enzyme–inhibitor complex is formed. (2) Time-dependent loss of enzyme activity is observed in agreement with the standard equation (eq 1) describing the reaction,[2] where K_i is the inhibitory constant, K_m the Michaelis constant, and [I] and [S] are the concentrations of inhibitor and substrate. (3) Nonspecific vs. active-site directed inactivation is distinguished by the decreased rate of enzyme inactivation in the presence of substrate; this "substrate protection" analysis also must satisfy the kinetic equation (eq 1).

$$1/k_{obsd} = \left[\frac{K_i[S]}{K_m[I]} + \frac{K_i}{k_{inact}}\right]\frac{1}{[I]} + \frac{1}{k_{inact}} \qquad (1)$$

Contemporary research in the design of a class of affinity labels termed mechanism-based inhibitors center on the utilization of the chemical mechanism of the catalytic event promoted by the enzyme for the activation of the inhibitor in the noncovalent complex of enzyme and inhibitor.[3] The activated inhibitor subsequently reacts with the enzyme resulting in covalent bond formation and enzyme inactivation, which may or may not be irreversible.

5-*p*-Benzoquinonyl-2′-deoxyuridine 5′-phosphate (**1a**) has been reported to be a possible mechanism-based inhibitor of thymidylate synthase.[4] The reductive alkylation reaction catalyzed by this enzyme is initiated by active-site thiol addition to carbon-6 of the α,β-unsaturated carbonyl moiety in the pyrimidine ring.[5] On this basis, compound **1** was designed as a potential mechanism-based inhibitor affording the reactive intermediate **2**, which would be ex-

Scheme I. Possible Mechanisms for the Inactivation of Thymidylate Synthase by 5-*p*-Benzoquinonyl-2′-deoxyuridine 5′-Phosphate (**1a**)



(1) Baker, B. R.; Lee, W. W.; Tong, E.; Ross, L. O. *J. Am. Chem. Soc.* **1961**, *83*, 3713–3714.
(2) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3248–3249.
(3) (a) Silverman, R. B.; Hoffman, S. J. *Med. Chem. Res. Rev.* **1984**, *4*, 415–447. (b) Walsh, C. *Tetrahedron* **1982**, *38*, 871–909. (c) Walsh, C. *Ann. Rev. Biochem.* **1984**, *53*, 493–535. (d) Abeles, R. H.; Maycock, A. *Acc. Chem. Res.* **1976**, *9*, 313–319. (e) Rando, R. R. *Acc. Chem. Res.* **1975**, *8*, 281–288.
(4) Maggiora, L.; Chang, C. T.-C.; Hasson, M. E.; Bigge, C. F.; Mertes, M. P. *J. Med. Chem.* **1983** *26*, 1028–1036.
(5) (a) Santi, D. V.; McHenry, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1855–1875. (b) Danenberg, P. V.; Langenbach, R. J.; Heidelberger, C. *Biochemistry* **1974**, *13*, 926–933. (c) Bellisario, R. L.; Maley, G. F.; Galivan, J. H.; Maley, F. *Proc. Natl. Acad. Sci, U.S.A.* **1076**, *73*, 1848–1852. (d) Maley, G. F.; Bellisario, R. L.; Guarino, D. U.; Maley, F. *J. Biol. Chem.* **1979**, *254*, 1288–1296, 1297–1300, 1301–1304.

0022-2623/86/1829-1714$01.50/0