J. Med. Chem. **1998**, *41*, 965−972

Intensely Potent Doxorubicin Analogues: Structure−Activity Relationship

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Received October 15, 1997

N-(5,5-Diacetoxypent-1-yl)doxorubicin (**1b**) is an intensely cytotoxic doxorubicin analogue that retains full potency against tumor cells that express elevated levels of P-glycoprotein and are resistant to doxorubicin. **1b** was designed to be hydrolyzed in the presence of carboxylate esterases to *N*-(5-oxypent-1-yl)doxorubicin, an aldehyde capable of existing in equilibrium with a cyclic carbinolamine. To investigate the structural determinants of potency for **1b**, we have prepared a series of chemically related compounds in which various ω-[bis(acetoxy)]alkyl or ω-[bis(acetoxy)]alkoxyalkyl groups are substituted at the 3′-amino position of the daunosamine sugar. These groups were selected to assess the effect of chain length, oxygen substitution, and carbinolamine ring size on analogue potency. The compounds were evaluated for their ability to inhibit the in vitro growth of the following cell lines: (a) Chinese hamster ovary (CHO) cells, (b) a CHO cell mutant 100-fold resistant to doxorubicin that expresses elevated levels of P-glycoprotein, (c) a murine ductal cell pancreatic adenocarcinoma (Panc 02), and (d) a murine mammary carcinoma (CA 755). The most potent members of the series were those that could form a straight chain aldehyde intermediate after esterase-mediated hydrolysis of the ω-bis(acetoxy) groups and give rise to 5- or 6-membered ring carbinolamines. Analogues capable of forming 7-, 8-, or 9-membered carbinolamines were markedly less active. The *N*-methyl derivative of **1b**, which cannot give rise to a cyclic carbinolamine, was 2 orders of magnitude less potent than **1b**. A branched chain analogue, **1f**, which contained a tertiary carbon atom adjacent to the ω-bis(acetoxy) groups, was also substantially less active than its nonbranched counterpart, **1a**. These findings suggest that the chain length of the 3′-amino substituents and the ability of the derived aldehydes to form 5- or 6-membered carbinolamines are critical determinants of biologic potency.

In an effort to develop doxorubicin analogues effective against tumors that express elevated levels of P-glycoprotein (glycoprotein 170, gp-170), we reported a series of derivatives in which an alkylating or latent alkylating group, R, was present at the 3′-amino position of the daunosamine sugar.[1−3] Our rationale in designing these compounds was that the aglycone moiety would intercalate between adjacent DNA base pairs and that the alkylating substituent would react covalently with nucleophilic groups within the DNA minor groove, thus preventing drug efflux from the cell. The most potent compound synthesized was *N*-(5,5-diacetoxypentyl)doxorubicin (**1b**; Scheme 1). **1b** was designed to undergo hydrolysis (Scheme 1) to the corresponding aldehyde, **3b**, by carboxylate esterases (E.C.3.1.1.1; carboxylate ester hydrolases), enzymes that are present in all tissues, including cells in culture.[4,5] Once formed, it was anticipated that the aldehyde would cyclize to the carbinolamine, **4b**. Loss of hydroxyl ion from **4b** would then generate the corresponding iminium ion, **5b**, which can theoretically react with a DNA nucleophile to form a covalently bonded adduct, **6b**. **1b** was 100-fold more potent than doxorubicin against a variety of human tumor cell lines in culture and retained full activity against variants of these cells that were resistant to doxorubicin and expressed elevated levels of gp-170.[2] **1b** was also effective against a cell line with altered topoisomerase II.[6]

To provide insight into the structural determinants of potency for **1b**, we prepared a series of analogues, **1a**−**l** (Schemes 1 and 2), in which various ω-substituted alkyl or alkoxyalkyl groups were present at the 3′-amino group of the daunosamine. Compounds **1a**−**i** (Scheme 1), which contain ω-bis(acetoxy) groups, were chosen to assess the effect of chain length, oxygen substitution, and chain branching on biologic potency. Hydrolysis of one of the ω-acetoxy groups of these compounds by carboxylate esterase should generate the hemiacetal intermediates, **2a**−**i**, which should spontaneously lose acetic acid to form the corresponding aldehydes, **3a**−**i**. Except for **3c**, these aldehydes can theoretically cyclize to form carbinolamines, **4a**−**i.** Aldehyde **3c** cannot form a carbinolamine because the 3′-amino group does not contain an exchangeable hydrogen atom. Compounds **1j**−**l**, which contain ω-substituents other than bis(acetoxy) groups (Scheme 2), were prepared for comparative studies. The *N*-(5-acetoxypent-1-yl) derivative, **1j**,[2] should give rise to an alcohol rather than an aldehyde upon ester group hydrolysis. The *N*-(pent-1-yl) derivative, **1k**,[2] unlike most other members of the series, is not susceptible to metabolic alteration by carboxylate esterases. **1l** was selected because it is a potential metabolite of **1a** derived by oxidation of the intermediate aldehyde, **3a**, by cellular oxidases or dehydrogenases.

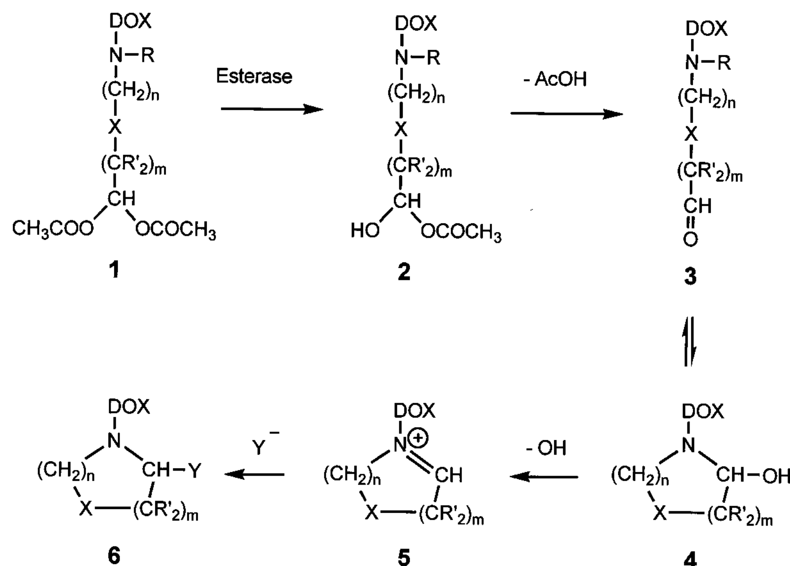
Chemistry

1a,b,d−**i** were synthesized by reductive condensation of doxorubicin with the appropriate aldehydes in the presence of $NaBH_3CN$ as shown in Scheme 3.

The bis(acetoxy) dialdehydes, **10a**−**e**, were prepared as shown in Scheme 4. Oxidation of the ω-alken-1-ols, **7a**−**e**, with tetrapropylammonium perruthenate (TPAP)[7]

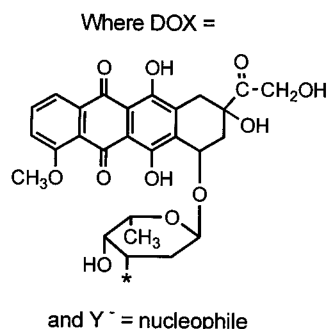
S0022-2623(97)00698-5 CCC: $15.00 © 1998 American Chemical Society
Published on Web 02/24/1998

Scheme 1



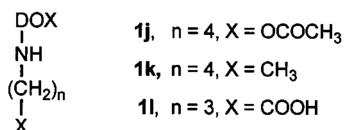
a, R = H, R' = H, n = 2, X = CH_2, m = zero
b, R = H, R' = H, n = 3, X = CH_2, m = zero
c, R = CH_3, R' = H, n = 3, X = CH_2, m = zero
d, R = H, R' = H, n = 4, X = CH_2, m = zero
e, R = H, R' = H, n = 6, X = CH_2, m = zero
f, R = H, R' = CH_3, n = 1, X = CH_2, m = 1
g, R = H, R' = H, n = 2, X = O, m = 1
h, R = H, R' = H, n = 2, X = O, m = 2
i, R = H, R' = H, n = 3, X = O, m = 2

Where DOX =

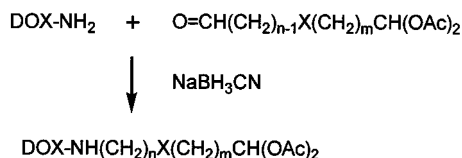


and Y^- = nucleophile

Scheme 2



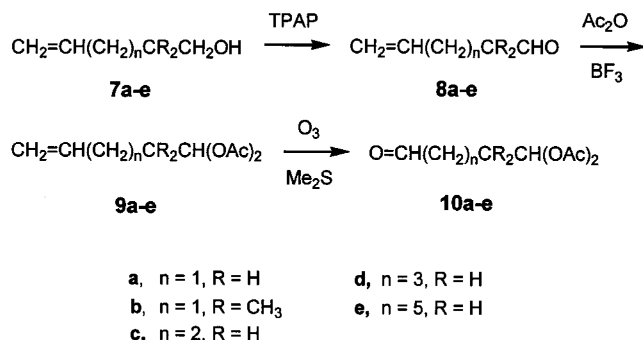
Scheme 3



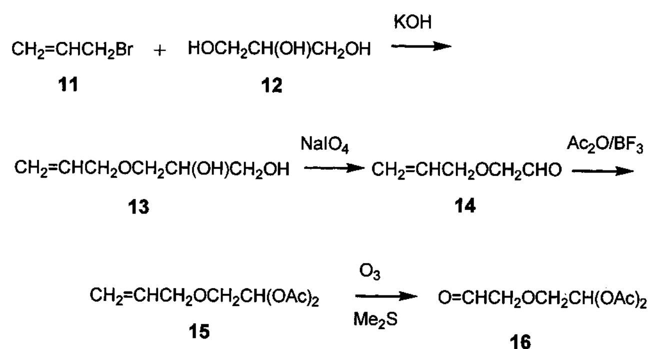
in CH_2Cl_2 afforded the corresponding aldehydes, **8a**–**e**. The aldehydes were converted to the corresponding bis(acetoxy) acetals, **9a**–**e**, by reaction with acetic anhydride in the presence of $BF_3 \cdot Et_2O$ as catalyst. Ozonolysis of **9a**–**e** at −78 °C followed by decomposition of the intermediate ozonides with dimethyl sulfide gave the dialdehyde monoacetals, **10a**–**e**, in excellent yield. Attempts to prepare shorter chain congeners (e.g., **10**, n = 0, R = H) were not successful because of the inherent instability of the required aldehyde precursors.

The oxygen isosteres **16** and **21a**,**b** were prepared as shown in Schemes 5 and 6. **1c** was synthesized by reductive alkylation of **1b** with formaldehyde. The free acid **1l** was prepared from the corresponding *tert*-butyl ester as shown in Scheme 7. Except for **1l**, all of the compounds were converted to the corresponding hydrochloride salts for biochemical and biological studies.

Scheme 4



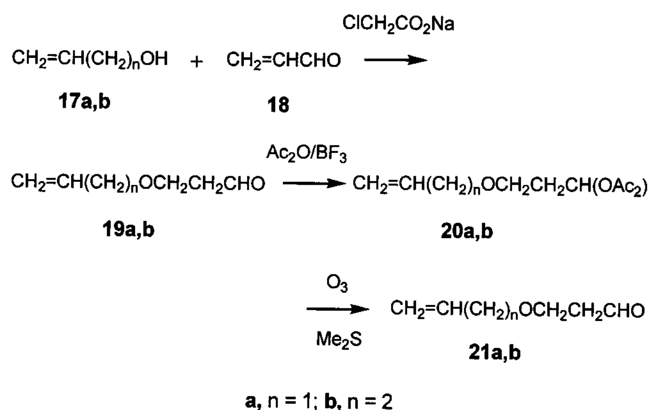
Scheme 5



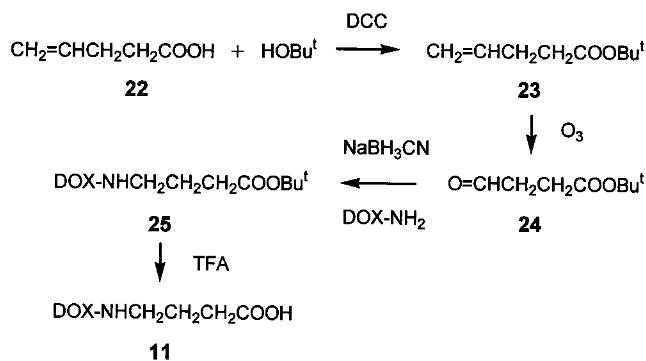
Enzyme Studies

Compounds **1a**–**i** were hydrolyzed with a half-life of less than 1 min when incubated in 0.05 M phosphate

Scheme 6



Scheme 7



buffer, pH 7.4, with carboxylate esterase (20 units of enzyme/μmol of substrate) at 37 °C. Under these conditions, no significant differences in the rates of hydrolysis were observed[8] (data not shown).

Cytotoxicity Studies

To assess comparative cytotoxicity, compounds **1a**–**l** were incubated for 6 days with the cell lines shown in Table 1. Chinese hamster ovary (CHO) cells were selected because of the availability of a variant with 100-fold resistance to doxorubicin (CHO/DOX) that expressed elevated levels of gp-170.[9] The CHO/DOX cell line is cross-resistant to vincristine and vinblastine.[9] The compounds were also screened against Panc 02, a mouse ductal cell pancreatic adenocarcinoma highly resistant to most antitumor drugs in vivo, and CA 755, a mouse mammary carcinoma.

The most potent members of the series were **1a**,**b**,**g**. These compounds were 50–600-fold more potent than doxorubicin at inhibiting the growth of the three wild-type cell lines. These compounds also showed pronounced cytotoxicity against the DOX/CHO cell line. The aldehydes derived from the esterase-mediated hydrolysis of **1a**,**b**,**g** can theoretically cyclize to 5-membered (**4a**) or 6-membered (**4b**,**g**) carbinolamines. **1d**,**e**,**h**,**i** were markedly less potent than **1a**,**b**,**g** against all cell lines. **1d**,**h** can theoretically give rise to 7-membered cyclic carbinolamines (**4d**,**h**). **1i**,**e** can similarly form an 8-membered (**4i**) and 9-membered (**4e**) cyclic carbinolamine, respectively. The *N*-methyl compound, **1c**, was 150–600-fold less growth inhibitory than its N-H counterpart, **1b**, against all cell lines; interestingly, **1c** can give rise to an open chain aldehyde but cannot form a cyclic carbinolamine. **1f**, which contains a

Table 1. Growth Inhibition of CHO Cells, a CHO Subline with Acquired Resistance to Doxorubicin (CHO/DOX), Murine Pancreatic Carcinoma Cells (Panc 02), and Murine Carcinoma 755 Cells (CA 755), after Incubation[a] with **1a**–**l** in Vitro

		IC_{50}[b,c] (10^{-10} M)			
compd	ring size[d]	CHO	CHO/DOX	Panc 02	CA 755
DOX	-	600	60000	650	140
1a	5	3	2	3	3
1b[f]	6	4	7	6	2
1c	none[e]	650	1850	1400	1300
1d	7	260	130	140	31
1e	9	2500	1700	1100	140
1f	5	1200	2900	950	900
1g	6	1	5	1	2
1h	7	550	960	600	nd
1i	8	1000	2100	800	900
1j[f]	none[e]	830	3500	670	1400
1k[f]	none[e]	570	4200	1000	330
1l	none[e]	2030	9500	2400	1500

[a] Exponentially growing cells were exposed to varying drug concentrations for 6 days at 37 °C. The cells were then fixed with formaldehyde, stained with crystal violet, and counted under an inverted phase-contrast microscope. [b] The drug concentration that inhibited cell growth by 50% compared to untreated control cultures. [c] At least seven drug concentrations ranging from 10^{-12} to 10^{-5} M were used to determine the IC_{50} values. Each value is the average of duplicate determinations. [d] Ring size of the putative carbinolamine intermediate. [e] Carbinolamine ring formation is not possible. [f] Data from ref 2. nd, not done.

tertiary carbon atom adjacent to the ω-bis(acetoxy) group, was approximately 300-fold less toxic than its straight chain counterpart, **1a**. **1j**–**l** were only modestly cytotoxic; these compounds are incapable of forming intermediate aldehydes.

1a,**b** were additionally evaluated by the National Cancer Institute against a battery of 50 human tumor cell lines in culture. The compounds showed a qualitatively similar pattern of activity with striking selectivity against the melanoma cell panel (the concentrations required for total growth inhibition ranged from 10^{-9} to 10^{-11} M; the average value for the 50-tumor cell battery was 10^{-8} M). **1a**,**b** also showed excellent selectivity against a few lung tumor cell lines and good inhibitory activity ($IC_{50} < 10^{-10}$) against most tumor cell types.

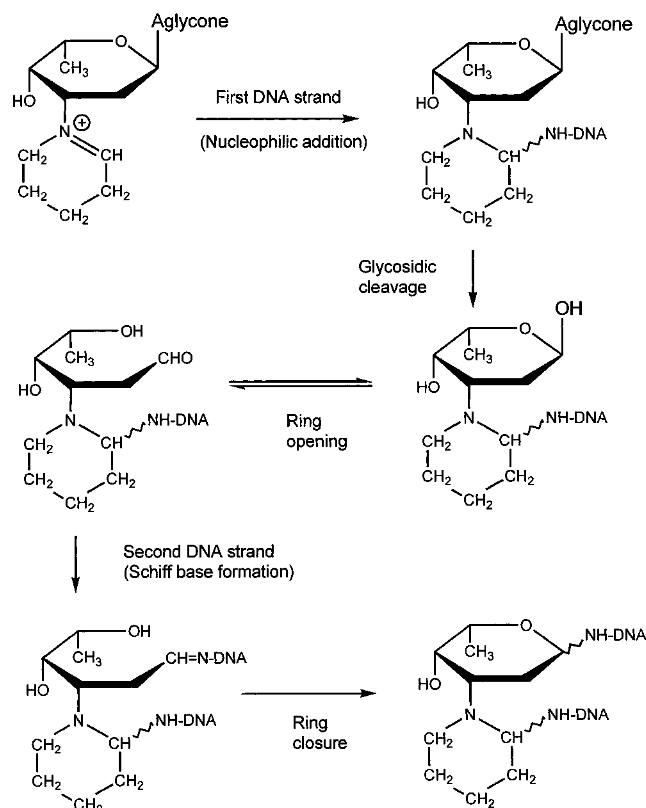
Discussion

Collectively, the above findings suggest that the ability of compounds of the general structure **1** to form aldehyde intermediates is a necessary, but not sufficient, condition for intense cytotoxicity. Thus, all members of the series that were intensely potent were capable of forming aldehyde intermediates. However, not all compounds that could form aldehyde intermediates were intensely potent. The structural property that correlated best with potency for this class of compounds was the ability of the putative aldehyde intermediates to form 5- or 6-membered cyclic carbinolamines. Except for **1f**, all compounds with this ability were intensely potent. **1f** was approximately 300-fold less toxic than its nonbranched counterpart **1a**. The most likely explanation for this difference is that the aldehyde derived from **1f** cannot readily cyclize because of unfavorable steric crowding between the two methyl groups and the hydroxyl group in the carbinolamine. The branched carbon center might also impede binding of the analogue to its critical target site. The presence of an oxygen atom in the side chain did not adversely affect activity. Thus **1g** was as potent as its methylene isostere, **1b**.

Compounds with the ability to give rise to 7-membered ring carbinolamines were much less potent than those that could form 5- or 6-membered ring carbinolamines. Compounds capable of forming 8- and 9- membered rings were even less potent. It is noteworthy that 5- and 6-membered alicyclic ring systems are abundant in nature, whereas 7-, 8-, and 9-membered ring systems are much less common, a difference generally attributed to the ring strain inherent in larger ring systems. Perhaps the most compelling evidence of a critical role for carbinolamines in the intense potency of certain members of this general class of compounds is the more than 100-fold greater potency of **1b** than its *N*-methyl counterpart, **1c**. Although both compounds are capable of forming aldehyde intermediates, only **1b** can give rise to a cyclic carbinolamine.

The biochemical mechanisms by which extremely potent members of this series exert their biological effects are not well-understood. However, we recently reported[6] that **1b** is highly effective at inducing endonucleolytic cleavage (i.e., apoptosis) in HL-60 cells and a subline resistant to doxorubicin characterized by altered topoisomerase II (HL-60/AMSA). We have also shown[6] that **1b** produces DNA–DNA cross-links in HL-60 and HL-60/AMSA cell lines and suggested that this mechanism contributes to its marked potency. However, the nature of the DNA–DNA cross-links is unclear. As noted above, it is likely that **1b** is hydrolyzed in cells to the aldehyde, **3b**, which then cyclizes to the carbinolamine, **4b**. From both theoretical and mechanistic considerations, it is probable that the carbinolamine loses hydroxide anion to form a cyclic iminium intermediate, **5b** (Scheme 1). Nucleophilic attack by a proximate base (such as the 2-amino group of a guanine residue) on **5b** would then yield a covalent adduct, **6b**. A similar mechanism has been suggested by Acton and colleagues[10] to account for the adduct formed between (cyanomorpholino)doxorubicin and DNA. Although this mechanism can adequately account for the primary covalent linkage between **1b** (and similarly constituted compounds) and DNA, the nature of the secondary covalent linkage, which leads to DNA–DNA cross-linking, is not readily apparent. One possibility is that the second linkage is formed after initial cleavage of the glycosidic linkage to form an aglycone and daunosamine sugar (Scheme 8). We hypothesize that as a consequence of DNA intercalation, the glycosidic linkage of **1b** becomes labilized. Support for this mechanism stems from our observation[11] that *N,N*-dimethyldoxorubicin forms a covalent linkage with a synthetic hexamer duplex [(d(TAGAGT)$_2$] through the *N,N*-dimethyldaunosamine moiety. Further support derives from crystallographic studies[12,13] of the adducts formed between various anthracyclines and deoxyoligonucleotides [such as daunomycin and d(CGTACG)] in which it has been shown that the glycosidic bond torsion angles have been altered and the glycosidic oxygens are often hydrogen-bonded to the 2-NH$_2$ groups of proximate guanine residues. Such effects are expected to facilitate hydrolytic cleavage of the aglycone–*O*-sugar linkage in molecules such as **1b**. A second possibility is that **1b** undergoes reductive cleavage at the intercalation site. In either event, the liberated sugar should exist in equilibrium with the corresponding ring-opened aldehyde which should be capable of condensing with the amino group of a proximate base (e.g., guanine) to form an imine. Ring closure would then regenerate the daunosamine sugar and complete the DNA–DNA cross-link. Though highly plausible, this mechanism remains to be demonstrated. Further studies addressing the intense potency of **1b** and structurally related compounds are in progress.

Scheme 8



Conclusions

The structure–activity relationships reported herein provide further insight into the mechanism of action of compounds of the general structure **1**. The most potent members of the series were those in which a ω-bis(acetoxy)methine group was linked to the 3′-amino group of the daunosamine sugar by an unbranched 3- or 4-unit spacer group. Such compounds can theoretically form aldehyde intermediates that can cyclize to 5- or 6-membered carbinolamines. Compounds that cannot form aldehyde intermediates or compounds predicted to have reduced ability to form cyclic carbinolamines were far less potent. Compounds with oxygen atoms in the side chain were similar in potency to their methylene counterparts. The findings are consistent with the hypothesis that the intense potency of certain compounds of the general structure **1** correlates with their ability to form 5- or 6-membered carbinolamines.

Experimental Section

Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded at ambient temperature on an IBM-Bruker model NR/200 AF spectrometer in the Fourier transform mode in $CDCl_3$ using Me_4Si as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are in hertz (Hz). NMR techniques used for

structural assignment included off-resonance decoupling, selective heteronuclear decoupling, homonuclear shift-correlated 2D-NMR (COSY), homonuclear shift-correlated 2D-NMR with a delay period to emphasize long-range or small coupling (COSYLR), and heteronuclear shift-correlated 2D-NMR with polarization transfer from ^{1}H to ^{13}C via J_{CH} (XH-CORR). Mass spectral analyses were conducted at the Midwest Center for Mass Spectrometry, Lincoln, NE. Samples were formulated in a 3-nitrobenzyl alcohol matrix and analyzed by fast-atom bombardment on a Kratos MS 50 TA instrument. All chemical reactions were conducted in dry glassware. Solvents were dried over freshly activated (300 °C, 1 h) molecular sieves (type 4A). The homogeneity of the products was determined by ascending TLC on silica-coated glass plates (silica gel 60F-254; Merck) using mixtures of $CHCl_3$−MeOH as the eluting solvent. Preparative separations (PLC) were performed on thick layers (20 cm × 20 cm × 2 mm) of the same adsorbent (60F-254; Merck) or by column chromatography on silica gel (Merck; 230−400 mesh) with mixtures of $CHCl_3$−MeOH as eluent. Organic extracts of aqueous solutions were dried over anhydrous Na_2SO_4. Solutions were concentrated under reduced pressure on a rotary evaporator. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN; where indicated only by the symbols of elements, the results were within ±0.4% of the theoretical values.

4-Penten-1-al (8a). Method A. Powdered molecular sieves (type 4A; 500 mg/mmol, 35 g) were added to a solution of 4-penten-1-ol (6.0 g, 7.2 mL, 69 mmol) and *N*-methylmorpholine *N*-oxide (1.22 g, 1.5 equiv, 104.5 mmol) in CH_2Cl_2 (150 mL). The mixture was stirred for 10 min at room temperature under a nitrogen atmosphere; then tetrapropylammonium perruthenate (TPAP)[6] (1.22 g, 3.45 mmol, 5 mol %) was added in one portion. The mixture turned green and gradually darkened as the reaction progressed. After 2 h (when the reaction was complete as evidenced by TLC) CH_2Cl_2 (150 mL) was added, and the mixture was filtered through a short pad of Celite. The solvent was evaporated, and the remaining liquid was chromatographed on a column of silica gel using $CHCl_3$−MeOH (98:2) as eluent to give **8a**[14] as an oil (4.4 g, 76%). ^{1}H NMR: δ 9.91 (t, *J* = 1 Hz, 1 H, H-1), 5.80 (m, 1 H, H-4), 5.21 (m, 2 H, H-5), 2.12 (m, 2 H, H-2), 1.95 (m, 2 H, H-3). ^{13}C NMR: 200.28 (C-1), 137.61 (C-4), 114.96 (C-5), 32.80 (C-2), 32.21 (C-3).

Method B. 5-Hexene-1,2-diol (2.2 g, 18.9 mmol) was added slowly, with stirring, over 10 min to a solution of $NaIO_4$ (4.1 g, 19.2 mmol) in H_2O (45 mL) at 4 °C. The mixture was left at room temperature for 2 h. EtOH (30 mL) was added, and the precipitated sodium salts were removed by filtration. The filtrate was concentrated and then partitioned between $CHCl_3$ (50 mL) and H_2O (20 mL). The organic layer was separated, dried, filtered, and evaporated. The crude product was chromatographed on a column of silica gel to give **8a** as a colorless oil (1.2 g, 75%). The spectral properties of the compound were identical with that prepared by method A.

5-Hexen-1-al (8c). The compound was prepared from 5-hexen-1-ol (1.67 g, 17 mmol) as described for **8a**. It was isolated as an oil[2] (0.98 g, 59%). ^{1}H NMR: δ 9.71 (t, *J* = 1 Hz, 1 H, H-1), 5.75 (m, 1 H, H-5), 5.04 (m, 2 H, H-6), 2.45 (m, 2 H, H-2), 2.08 (m, 2 H, H-4), 1.72 (m, 2 H, H-3). ^{13}C NMR: 201.95 (C-1), 137.18 (C-5), 115.07 (C-6), 42.67 (C-2), 32.55 (C-4), 20.75 (C-3).

6-Hepten-1-al (8d). The compound was prepared from 6-hepten-1-ol[15] (1.9 g, 17 mmol) as described for **8a**. It was isolated as an oil[16] (1.7 g, 90%). ^{1}H NMR: δ 9.62 (s, 1 H, H-1), 5.83 (m, 1 H, H-6), 4.92 (m, 2 H, H-7), 2.41 (t, *J* = 7 Hz, 2 H, H-2), 1.74 (m, 2 H, H-3), 1.51 (m, 2 H, H-5), 1.35 (m, 2 H, H-4). ^{13}C NMR: 202.51 (C-1), 138.04 (C-6), 114.67 (C-7), 43.44 (C-2), 33.43 (C-5), 28.13 (C-3), 24.81 (C-4).

8-Nonen-1-al (8e). The compound was prepared from 8-nonen-1-ol[17] (2.4 g, 17 mmol) as described for **8a**. It was isolated as an oil[18] (2.1 g, 89%). ^{1}H NMR: δ 9.65 (s, 1 H, H-1), 5.72 (m, 1 H, H-8), 4.95 (m, 2 H, H-9), 2.43 (t, *J* = 5 Hz, 2 H, H-2), 2.12 (m, 2 H, H-7), 1.54 (m, 2 H, H-3), 1.3−1.4 (m, 6 H, H-4, H-5, H-6). ^{13}C NMR: 200.54 (C-1), 138.82 (C-8), 114.11 (C-9), 35.01 (C-2), 34.24 (C-7), 28.83 (C-3), 28.74 (C-6), 23.92 (C-4), 23.24 (C-5).

2,2-Dimethyl-4-pentene 1,1-Diacetate (9b). 2,2-Dimethyl-4-penten-1-al (2.5 g, 22 mmol) was added dropwise, with stirring, over 5 min at ambient temperature, to a solution of acetic anhydride (2.5 mL, 26.4 mmol) and BF_3·Et_2O (0.2 mL) in anhydrous Et_2O (5 mL). The mixture was stirred for 10 min, washed successively with 25% NaOAc solution (10 mL) and H_2O (10 mL × 2), and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was chromatographed on a column of silica gel using $CHCl_3$−MeOH (97:3) as eluent to give **9b** as an oil (4.4 g, 94%). ^{1}H NMR: δ 6.52 (s, 1 H, H-1), 5.75 (m, 1 H, H-4), 4.95 (m, 2 H, H-5), 2.05 (s, 6 H, 2 × $OCOCH_3$), 2.02 (m, 2 H, H-3), 1.85 (s, 6 H, 2 × CH_3). ^{13}C NMR: 168.63 (O*C*OCH_3), 133.46 (C-4), 117.65 (C-5), 93.55 (C-1), 41.43 (C-3), 37.42 (C-2), 21.01 (*C*H_3), 20.34 (OCO*C*H_3). Anal. ($C_{11}H_{18}O_4$) C, H.

5-Hexene 1,1-Diacetate (9c). The compound was prepared from 5-hexen-1-al (5.0 g, 51 mmol) as described for **9b**. It was isolated as an oil.[2] The yield was 9.6 g (94%). ^{1}H NMR: δ 6.83 (t, *J* = 5 Hz, 1 H, H-1), 5.58 (m, 1 H, H-5), 5.02 (m, 2 H, H-6), 2.12 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2), 1.85 (m, 2 H, H-4), 1.50 (m, 2 H, H-3). ^{13}C NMR: 168.42 (O*C*OCH_3), 137.43 (C-5), 114.81 (C-6), 89.92 (C-1), 32.82 (C-2), 32.14 (C-4), 22.23 (C-3), 20.32 (OCO*C*H_3). Anal. ($C_{10}H_{16}O_4$) C, H.

6-Heptene 1,1-Diacetate (9d). The compound was prepared from 6-hepten-1-al (4.0 g, 35.7 mmol) as described for **9b**. It was isolated as an oil. The yield was 7.14 g (95%). ^{1}H NMR: δ 6.87 (t, *J* = 5 Hz, 1 H, H-1), 5.83 (m, 1 H, H-6), 4.98 (m, 2 H, H-7), 2.15 (dt, *J* = 5.0, 1.0 Hz, 2 H, H-2), 2.02 (s, 6 H, 2 × $OCOCH_3$), 1.90 (m, 2 H, H-5), 1.64 (m, 4 H, H-3, H-4). ^{13}C NMR: 168.54 (O*C*OCH_3), 137.93 (C-6), 114.92 (C-7), 89.86 (C-1), 33.43 (C-2), 33.14 (C-5), 24.52 (C-3), 21.21 (C-4), 20.22 (OCO*C*H_3). Anal. ($C_{11}H_{18}O_4$) C, H.

8-Nonene 1,1-Diacetate (9e). The compound was prepared from 8-nonen-1-al (7.0 g, 50 mmol) as described for **9b**. It was isolated as an oil. The yield was 11.75 g (97%). ^{1}H NMR: δ 6.82 (t, *J* = 5.6 Hz, 1 H, H-1), 5.75 (m, 1 H, H-8), 4.95 (m, 2 H, H-9), 2.02 (s, 6 H, 2 × $OCOCH_3$), 1.98 (m, 2 H, H-7), 1.75 (m, 2 H, H-2), 1.54 (m, 2 H, H-4), 1.35 (m, 6 H, H-3, H-5, H-6). ^{13}C NMR: 168.84 (O*C*OCH_3), 138.83 (C-8), 114.12 (C-9), 90.43 (C-1), 34.92 (C-2), 34.15 (C-7), 28.84 (C-3), 28.71 (C-6), 23.84 (C-4), 23.21 (C-5), 20.64 (OCO*C*H_3). Anal. ($C_{13}H_{22}O_4$) C, H.

4-Oxobutane 1,1-Diacetate (10a). A solution of 5-pentene 1,1-diacetate (**9a**)[2] (4.65 g, 25 mmol) in CH_2Cl_2 (50 mL) was placed in a long cylindrical gas absorption vessel with an inlet dispersion tube extending to the base. The vessel was cooled to −70 °C in a dry ice−acetone mixture, and ozone was introduced. Ozonization was continued until reaction was complete (until the mixture turned blue as a result of formation of the ozonide, approximately 20 min). Me_2S (7.3 mL, 100 mmol, 4 equiv) was added, and the mixture was stirred overnight to reduce the ozonide to the corresponding aldehyde. The excess Me_2S was evaporated, and the residue was chromatographed on a column of silica gel (CH_2Cl_2) to give **10a** as an oil (4.04 g, 86%). ^{1}H NMR: δ 9.83 (t, *J* = 1.2 Hz, 1 H, H-4), 6.75 (t, *J* = 5.2 Hz, 1 H, H-1), 2.66 (dt, *J* = 5.5, 1.2 Hz, 2 H, H-3), 2.14 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2). ^{13}C NMR: 202.05 (C-4), 167.83 (O*C*OCH_3), 89.85 (C-1), 42.91 (C-3), 34.53 (C-2), 20.45 (OCO*C*H_3). Anal. ($C_8H_{12}O_5$) C, H.

2,2-Dimethyl-4-oxobutane 1,1-Diacetate (10b). The compound was prepared from **9b** (5.35 g, 25 mmol) as described for **10a**. It was obtained as an oil (4.68 g, 87%). ^{1}H NMR: δ 9.85 (t, *J* = 1.2 Hz, 1 H, H-4), 6.65 (t, *J* = 5 Hz, 1 H, H-1), 2.08 (s, 6 H, 2 × $OCOCH_3$), 2.01 (m, 2 H, H-3), 1.85 (s, 6 H, 2 × *C*H_3). ^{13}C NMR: 201.05 (C-4), 168.72 (O*C*OCH_3), 89.65 (C-1), 42.80 (C-3), 37.42 (C-2), 21.50 (CH_3), 20.35 (OCO*C*H_3). Anal. ($C_{10}H_{16}O_5$) C, H.

5-Oxopentane 1,1-Diacetate (10c). The compound was prepared from **9c** (5.0 g, 3.5 mL, 25 mmol) in CH_2Cl_2 (50 mL) as described for **10a**. It was obtained as a syrup[2] (4.29 g, 85%). ^{1}H NMR: δ 9.83 (t, *J* = 1 Hz, 1 H, H-5), 6.75 (t, *J* = 5 Hz, 1

H, H-1), 2.66 (dt, $J = 5.5$, 1.0 Hz, 2 H, H-4), 2.14 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2), 1.85 (m, 2 H, H-3). ^{13}C NMR: 201.43 (C-5), 168.72 (O*C*OCH$_3$), 89.61 (C-1), 42.86 (C-4), 32.10 (C-2), 20.54 (OCO*C*H$_3$), 15.62 (C-3). Anal. ($C_9H_{14}O_5$) C, H

6-Oxohexane 1,1-Diacetate (10d). The compound was prepared from **9d** (5.35 g, 25 mmol) as described for **10a**. It was obtained as an oil (4.71 g, 87%). ^1H NMR: δ 9.83 (t, $J = 1$ Hz, 1H, H-6), 6.75 (t, $J = 5$ Hz, 1 H, H-1), 2.66 (dt, $J = 5.5$, 1.0 Hz, 2 H, H-5), 2.14 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2), 1.85 (m, 2 H, H-3), 1.82 (m, 2 H, H-4). ^{13}C NMR: 201.43 (C-6), 168.72 (O*C*OCH$_3$), 89.61 (C-1), 42.65 (C-5), 32.10 (C-2), 20.54 (OCO*C*H$_3$), 15.62 (C-3), 15.60 (C-4). Anal. ($C_{10}H_{16}O_5$) C, H.

8-Oxooctane 1,1-Diacetate (10e). The compound was prepared from the acetate **9e** (6.05 g, 2.48 g, 25 mmol) in CH_2Cl_2 (50 mL) as described for **10a**. It was obtained as oil (5.12 g, 84%). ^1H NMR: δ 9.83 (t, $J = 1.1$ Hz, 1 H, H-8), 6.75 (t, $J = 5$ Hz, 1 H, H-1), 2.66 (dt, $J = 5.5$, 1.1 Hz, 2 H, H-7), 2.14 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2), 1.85 (m, 2 H, H-3), 1.82–1.75 (m, 6 H, H-4, H-5, H-6). ^{13}C NMR: 201.50 (C-8), 168.70 (O*C*OCH$_3$), 89.66 (C-1), 42.88 (C-7), 32.15 (C-2), 20.58 (OCO*C*H$_3$), 15.62 (*C-3), 15.60 (*C-4), 15.46 (*C-5), 15.00 (*C-5) (*assignments may be interchanged). Anal. ($C_{12}H_{20}O_5$) C, H.

(Allyloxy)acetaldehyde (14). 3-(Allyloxy)propane-1,2-diol (13)[19] (2.5 g, 18.9 mmol) was added, with stirring, over 10 min to a solution of $NaIO_4$ (4.1 g, 19.2 mmol) in H_2O (45 mL) maintained at 4 °C. The mixture was then allowed to stand at room temperature for 2 h. EtOH (30 mL), was added and the precipitated sodium salts were removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was partitioned between $CHCl_3$ (50 mL) and H_2O (20 mL). The organic layer was separated, dried, filtered, and evaporated. The residue was chromatographed on a column of silica gel using $CHCl_3$–MeOH (96:4) as eluent. **14** was obtained as a syrup (1.32 g, 70%). ^1H NMR: δ 9.73 (t, $J = 1$ Hz, 1 H, H-1), 5.95 (m, 1 H, H-2′), 5.37 (m, 2 H, H-3′), 4.05 (m, 4 H, H-1′, H-2). ^{13}C NMR: 199.86 (C-1), 133.32 (C-2′), 117.02 (C-3′), 74.65 (C-2), 71.64 (C-1′).

2-(Allyloxy)ethane 1,1-Diacetate (15). **14** (1.32 g, 13.2 mmol) was added dropwise, with stirring over 5 min at room temperature, to a solution of acetic anhydride (1.5 mL, 15.8 mmol) and $BF_3 \cdot Et_2O$ (0.1 mL) in Et_2O (5 mL). The mixture was stirred for 10 min, then washed successively with 25% NaOAc solution (5 mL) and H_2O (5 mL × 2), and dried. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (97:3) as eluent to give **15** as an oil (2.55 g, 95%). ^1H NMR: δ 6.82 (t, $J = 5$ Hz, 1 H, H-1), 5.75 (m, 1 H, H-2′), 5.15 (m, 2 H, H-3′), 3.98 (m, 2 H, H-1′), 3.55 (d, $J = 5$ Hz, 2 H, H-2), 2.0 (s, 6 H, 2 × $OCOCH_3$). ^{13}C NMR: 168.3 (O*C*OCH$_3$), 133.74 (C-2′), 117.21 (C-3′), 87.34 (C-1), 72.04 (C-1′), 68.53 (C-2), 20.31 (OCO*C*H$_3$).

[(2,2-Diacetoxyethyl)oxy]acetaldehyde (16). **15** (2.55 g, 12.6 mmol) in CH_2Cl_2 (25 mL) was ozonized as described for **10a**. Me_2S (3.7 mL, 50.5 mmol, 4 equiv) was to reduce the ozonide. The crude product was purified on a column of silica gel (CH_2Cl_2 as eluent) to give **16** as a viscous oil (2.21 g, 86%). ^1H NMR: δ 9.62 (m, 1 H, $J = 1$ Hz, H-1), 6.84 (t, 1 H, $J = 5.2$ Hz, H-2′), 4.23 (d, 2 H, $J = 1$ Hz, H-2), 3.86 (d, 2 H, $J = 5.2$ Hz, H-1′), 2.01 (s, 6 H, 2 × $OCOCH_3$). ^{13}C NMR: 199.94 (C-1), 168.35 (O*C*OCH$_3$), 87.37 (C-2′), 72.32 (C-2), 68.51 (C-1′), 20.53 (OCO*C*H$_3$). Anal. ($C_8H_{12}O_6$) C, H.

3-(Allyloxy)propionaldehyde (19a). A solution of allyl alcohol (**17a**) (30 mL, 25.6 g, 0.44 mol), monochloroacetic acid (3.0 g, 0.032 mol), and sodium hydroxide (1.27 g, 0.032 mol) in H_2O (5 mL) was added dropwise, with stirring over 10 min, to acrolein (**18**) (45 mL, 37.8 g, 0.67 mol) contained in a 250-mL flask. Acetic acid (12 mL, 0.21 mol) was added, and the reaction mixture was maintained at 40 °C for 40 h. After cooling to room temperature, the mixture was washed with H_2O (50 mL × 3), and the organic layer was dried over anhydrous Na_2SO_4. The solution was concentrated under aspirator vacuum at 40 °C to remove volatile byproducts. The crude product was obtained as a viscous oil. For subsequent reaction, batches of the crude product (4 g) were purified by column chromatography on silica gel using CH_2Cl_2 as eluent. The yield of pure **19a** was 69%. ^1H NMR: δ 9.82 (t, 1 H, $J = 1$ Hz, H-1), 5.82 (m, 1H, H-2′), 5.21 (m, 2 H, H-3′), 3.93 (tt, 2 H, $J = 3$, 1 Hz, H-1′), 3.82 (t, 2 H, $J = 4$ Hz, H-3), 2.61 (tt, 2 H, $J = 4$, 1 Hz, H-2). ^{13}C NMR: 202.12 (C-1), 134.24 (C-2′), 116.24 (C-3′), 71.43 (C-1′), 64.34 (C-3), 34.12 (C-2). Anal. ($C_6H_{10}O_2$) C, H.

3-(4-Butenyloxy)propionaldehyde (19b). The compound was prepared from 4-buten-1-ol **(17b)** (15.0 g, 0.21 mol) and acrolein (**18**) (21 mL, 17.6 g, 0.32 mol) in the presence of monochloroacetic acid (1.18 g, 0.0125 mol), NaOH (0.5 g, 0.0125 mol), H_2O (3 mL), and acetic acid (6 mL, 0.11 mol) as described for **19a**. The yield of pure product was 64%. ^1H NMR: δ 9.79 (t, 1 H, $J = 1$ Hz, H-1), 5.80 (m, 1 H, H-3′), 5.11 (m, 2 H, H-4′), 3.83 (t, 2 H, $J = 3$ Hz, H-3), 3.52 (t, 2 H, $J = 4$ Hz, H-1′), 2.69 (tt, 2 H, $J = 4$, 1 Hz, H-2), 2.32 (m, 2 H, H-3′). Anal. ($C_7H_{12}O_2$) C, H.

3-(Allyloxy)propane 1,1-Diacetate (20a). The compound was prepared from **19a** (5.0 g, 5.2 mL, 44 mmol) as described for **15**. The crude product was purified by column chromatography on silica gel (96:4 $CHCl_3$–MeOH) to give **20a** (8.94 g, 94%). ^1H NMR: δ 6.92 (t, 1 H, $J = 4$ Hz, H-1), 5.85 (m, 1 H, H-2′), 5.23 (m, 2 H, H-3′), 3.95 (tt, 2 H, $J = 3$, 1 Hz, H-1′), 3.44 (t, 2 H, $J = 4$ Hz, H-3), 2.01 (s, 6 H, 2 × $OCOCH_3$), 1.95 (tt, 2 H, $J = 4$, 2 Hz, H-2). ^{13}C NMR: 168.02 (O*C*OCH$_3$), 134.44 (C-2′), 116.43 (C-3′), 88.42 (C-1), 71.41 (C-1′), 64.76 (C-3), 33.25 (C-2), 20.26 (OCO*C*H$_3$). Anal. ($C_{10}H_{16}O_5$) C, H.

3-(4-Butenyloxy)propane 1,1-Diacetate (20b). The compound was prepared from 3-(4-butenyloxy)propionaldehyde **(19b)** (1.68 g, 13.2 mmol) as described for **15**. The crude product was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 98:2, as eluent) to give **20b** as an oil (2.6 g, 86%). ^1H NMR: δ 6.92 (t, 1 H, $J = 4$ Hz, H-1), 5.82 (m, 1 H, H-3′), 5.10 (m, 2 H, H-4′), 3.53 (t, 2 H, $J = 3$, H-3), 3.48 (t, 2 H, $J = 4$ Hz, H-1′), 2.35 (m, 2 H, H-2′), 2.07 (s, 6 H, 2 × $OCOCH_3$), 2.02 (m, 2 H, H-2). ^{13}C NMR: 168.77 (O*C*OCH$_3$), 135.13 (C-3′), 116.32 (C-4′), 808.90 (C-1), 70.37 (C-3), 64.76 (C-1′), 34.08 (*C-2), 33.60 (*C-2′), 20.77 (OCO*C*H$_3$) (*assignments may be interchanged). Anal. ($C_{11}H_{18}O_5$) C, H.

[(3,3-Diacetoxypropyl)oxy]acetaldehyde (21a). The compound was prepared by ozonolysis of **20a** (5.4 g, 25 mmol) as described for **10a**. The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to give the aldehyde as a colorless oil (4.58 g, 84%). ^1H NMR: δ 9.63 (t, 1 H, $J = 1$ Hz, H-1), 6.74 (t, 1 H, $J = 4.2$ Hz, H-3′), 4.27 (d, 2 H, $J = 1$ Hz, H-2), 3.45 (t, 2 H, $J = 4.2$ Hz, H-1′), 2.01 (s, 6 H, 2 × $OCOCH_3$), 1.95 (tt, 2 H, $J = 4$, 2 Hz, H-2′). ^{13}C NMR: 199.94 (C-1), 168.35 (O*C*OCH$_3$), 88.37 (C-3′), 72.32 (C-2), 64.66 (C-1′), 33.35 (C-2′), 20.36 (OCO*C*H$_3$). Anal. ($C_9H_{14}O_6$) C, H.

[(3,3-Diacetoxypropyl)oxy]propionaldehyde (21b). The compound was prepared by ozonolysis of **20b** (2.0 g, 8.6 mmol) in CH_2Cl_2 (20 mL) as described for **10a**. The crude product was purified by column chromatography on silica gel (CH_2Cl_2) to give the aldehyde **21b** (1.53 g, 76%) as a colorless oil. ^1H NMR: δ 9.78 (t, 1 H, $J = 1$ Hz, H-1), 6.88 (t, 1 H, $J = 3$ Hz, H-3′), 3.75 (m, H-3), 3.54 (tt, 2 H, $J = 3.1$, 0.72 Hz, H-1′), 2.67 (m, 2 H, H-2), 2.08 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2′). ^{13}C NMR: 201.08 (C-1), 168.68 (O*C*OCH$_3$), 88.35 (C-3′), 65.31 (C-3), 64.28 (C-1′), 34.48 (C-2), 33.15 (C-2′), 20.55 (OCO*C*H$_3$). Anal. ($C_{10}H_{16}O_6$) C, H.

***tert*-Butyl 5-Pentenoate (23).** A solution of 4-pentenoic acid **(22)** (4.0 g, 40 mmol), 4-(*N,N*-dimethylamino)pyridine (80 mg), and *tert*-butyl alcohol (8.9 g, 11.32 mL, 120 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C. Dicyclohexylcarbodiimide (9.0 g, 44 mmol) was added, and the mixture was stirred for 5 min at 0 °C. It was then warmed to room temperature and stirred overnight. The dicyclohexylurea which formed was removed by filtration, and the filtrate was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (75 mL), and the solution was washed sequentially with ice-cold 0.1 N HCl (2 × 75 mL), saturated $NaHCO_3$ (75 mL), and water (2 × 75 mL). The crude product obtained by evaporation of solvent was purified by column chromatography on silica gel to give **23** as an oil (4.24 g, 68%). ^1H NMR: δ 5.70 (m, 1 H, H-4), 4.80 (m, 2 H, H-5),

2.25 (m, 4 H, H-2, H-3), 1.38 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR: 172.03 (C-1), 136.65 (C-4), 114.97 (C-5), 79.82 ($C(CH_3)_3$), 34.45 (C-3), 28.87 (C-2), 27.83 ($C(CH_3)_3$). Anal. ($C_9H_{16}O_2$) C, H.

***tert*-Butyl 4-Oxobutanoate (24).** The compound was prepared by ozonolysis of **23** (2.0 g, 12.8 mmol) as described for **10a**. The crude product was purified by column chromatography on silica gel to give **24** as an oil (1.48 g, 73%). ^{1}H NMR: δ 9.70 (t, 1 H, H-4), 2.45 (m, 2 H, H-3), 2.60 (t, 2 H, H-2), 1.35 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR: 200.17 (C-4), 171.22 (C-1), 80.51 ($C(CH_3)_3$), 38.42 (C-3), 27.73 ($C(CH_3)_3$), 27.60 (C-2). Anal. ($C_8H_{14}O_3$) C, H.

***N*-(4,4-Diacetoxybut-1-yl)doxorubicin Hydrochloride (1a).** A stirred solution of doxorubicin hydrochloride (20 mg, 0.035 mmol) and 4-oxobutane 1,1-diacetate **(10a)** (13.2 mg, 2 equiv, 0.07 mmol) in CH_3CN-H_2O (2:1) (5 mL) was treated with a solution of $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol). The mixture was stirred under a nitrogen atmosphere at room temperature in the dark for 1 h. When reaction was complete (as evidenced by TLC of a 5-μL aliquot), the solution was diluted with H_2O (8 mL) and then extracted repeatedly (10 × 10 mL) with $CHCl_3-MeOH$ (5:1). The combined extracts were dried and evaporated to give a red film (16 mg) which was purified by preparative TLC using $CHCl_3-MeOH$ (10:1) as eluent. The product was suspended in H_2O (1 mL) and acidified to pH 5 by dropwise addition of 0.05 N hydrochloride (approximately 0.5 mL). The solution was lyophilized to afford the title compound (10.2 mg, 39%). It was stored under nitrogen in a tightly stoppered vessel at −78 °C in the dark. TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.59. ^{1}H NMR (free base): δ 8.05 (dd, J = 8.1, 0.85 Hz, 1 H, H-1), 7.90 (t, J = 8.1 Hz, 1 H, H-2), 7.42 (dd, J = 8.1, 0.85 Hz, 1 H, H-3), 6.75 (t, J = 5.45 Hz, 1 H, H-4″), 5.52 (bs, 1 H, H-1′), 5.31 (bs, 1 H, H-7), 4.72 (s, 2 H, H-14), 4.05 (s, 3 H, 4-OCH_3), 3.95 (m, 1 H, H-5′), 3.61 (m, 1 H, H-4′), 3.32 (d, J = 16.2 Hz, 1 H, H-10a), 3.03 (d, J = 16.2 Hz, 1 H, H-10b), 2.81 (m, 1 H, H-3′), 2.60 (m, 2 H, H-1″), 2.44 (m, 1 H, H-8a), 2.22 (m, 1 H, H-8b), 2.02 (s, 6 H, 2 × $OCOCH_3$), 1.75 (m, 2 H, H-2′a), 1.75 (m, 1 H, H-3″), 1.71 (m, 1 H, H-2′b), 1.42 (m, 2 H, H-2″), 1.35 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 716 (M + H)$^+$. HRMS calcd for ($C_{35}H_{42}NO_{15}$) 716.2554, found 716.2554.

***N*-(5,5-Diacetoxypent-1-yl)doxorubicin Hydrochloride (1b).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), 5-oxopentane 1,1-diacetate (**10c**) (14 mg, 2 equiv, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL) as previously reported.[2] The yield was 10.3 mg (38%). TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.60.

***N*-Methyl-*N*-(5,5-diacetoxypent-1-yl)doxorubicin Hydrochloride (1c).** Formalin (3.3 μL of a 37% aqueous solution, 41 μmol) was added to a solution of *N*-(5,5-diacetoxypentyl)doxorubicin hydrochloride **(1b)** (3.0 mg, 4.1 μmol) in CH_3CN-H_2O (2:1) (2 mL). The mixture was stirred for 30 min at room temperature. $NaBH_3CN$ (1 M) in THF (4.1 μL, 4.1 μmol, 0.67 equiv) was added and the mixture was stirred for an additional 24 h. The crude product was isolated as described for **1a** and purified by preparative TLC on silica gel. The yield was 1 mg (33%). TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.60. ^{1}H NMR (free base): δ 7.99 (d, J = 8 Hz, 1 H, H-1), 7.82 (t, J = 8 Hz, 1 H, H-2), 7.36 (dd, J = 8, 1 Hz, 1 H, H-3), 6.65 (t, J = 5.5 Hz, 1 H, H-5″), 5.55 (t, 1 H, H-1′), 5.26 (bs, 1 H, H-7), 4.72 (s, 2 H, H-14), 4.05 (s, 3 H, 4-OCH_3), 3.96 (m, 1 H, H-5′), 3.63 (m, 1 H, H-4′), 3.21 (d, J = 16 Hz, 1 H, H-10a), 2.95 (d, J = 16 Hz, 1 H, H-10b), 2.61 (m, 1 H, H-3′), 2.50 (m, 2 H, H-1″), 2.30 (s, 3 H, N-CH_3), 2.2 (m, 2 H, H-8a, H-8b), 2.00 (s, 6 H, 2 × $OCOCH_3$), 1.74 (m, 4 H, H-2′a, H-2′b, H-4″), 1.35 (m, 2 H, H-3″), 1.30 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 744 (M + H)$^+$. HRMS calcd for ($C_{37}H_{46}NO_{15}$) 744.2867, found 744.2889.

***N*-(6,6-Diacetoxyhexyl)doxorubicin Hydrochloride (1d).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), 6-oxohexane 1,1-diacetate (**10d**) (15 mg, 2 equiv, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL) as described for **1a**. The yield was 10.75 mg (39%). ^{1}H NMR (free base): δ 8.03 (dd, J = 8.2, 0.9 Hz, 1 H, H-1), 7.84 (t, J = 8.2 Hz, 1 H, H-2), 7.37 (dd, J = 8.2, 0.9 Hz, 1 H, H-3), 6.76 (t, J = 5.46 Hz, 1 H, H-6″), 5.52 (t, J = 1 Hz, 1 H, H-1′), 5.4 (bs, 1 H, H-7), 4.75 (s, 2 H, H-14), 4.14 (s, 3 H, 4-OCH_3), 3.97 (m, 1 H, H-5′), 3.63 (m, 1 H, H-4′), 3.25 (d, J = 16 Hz, 1 H, H-10a), 2.96 (d, J = 16 Hz, 1 H, H-10b), 2.85 (m, 1 H, H-3′), 2.66 (m, 2 H, H-1″), 2.36 (m, 1 H, H-8a), 2.23 (m, 1 H, H-8b), 2.05 (s, 6 H, 2 × $OCOCH_3$), 1.82 (m, 1 H, H-2′a), 1.78 (m, 2 H, H-5″), 1.75 (m, 1 H, H-2′b), 1.65 (m, 2 H, H-2″), 1.40 (m, 2 H, H-3″), 1.39 (m, 2 H, H-4″), 1.32 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 744 (M + H)$^+$. HRMS calcd for ($C_{37}H_{46}NO_{15}$) 744.2867, found 744.2867.

***N*-(8,8-Diacetoxyoct-1-yl)doxorubicin Hydrochloride (1e).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), 8-oxooctane-1,1-diacetate (**10e**) (17.1 mg, 2 equiv, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL) as described for (**1a**). TLC: silica gel, $CHCl_3$-MeOH, 10:1; R_f = 0.60). The yield was 11.72 mg (41%). ^{1}H NMR (free base): δ 8.05 (dd, J = 8.2, 0.9 Hz, 1 H, H-1), 7.85 (t, J = 8.2 Hz, 1 H, H-2), 7.40 (dd, J = 8.2, 0.9 Hz, 1 H, H-3), 6.72 (t, J = 5.46, 1 H, H-8″), 5.55 (t, J = 1 Hz, 1 H, H-1′), 5.35 (bs, 1 H, H-7), 4.71 (s, 2 H, H-14), 4.16 (s, 3 H, 4-OCH_3), 3.97 (m, 1 H, H-5′), 3.65 (m, 1 H, H-4′), 3.28 (d, J = 16 Hz, 1 H, H-10a), 2.98 (d, J = 16 Hz, 1 H, H-10b), 2.85 (m, 1 H, H-3′), 2.68 (m, 2 H, H-1″), 2.35 (m, 1 H, H-8a), 2.25 (m, 1 H, H-8b), 2.04 (s, 6 H, 2 × $OCOCH_3$), 1.85 (m, 1 H, H-2′a), 1.75 (m, 2 H, H-7″), 1.76 (m, 1 H, H-2′b), 1.68 (m, 2 H, H-2″), 1.41 (m, 2 H, H-3″), 1.40 (m, 6 H, H-4″, H-5″), 1.39 (m, 2 H, H-6″), 1.35 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 772 (M + H)$^+$. HRMS calcd for ($C_{39}H_{50}NO_{15}$) 772.3180, found 772.3180.

***N*-(4,4-Diacetoxy-3,3-dimethylbut-1-yl)doxorubicin Hydrochloride (1f).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), 2,2-dimethyl-4-oxobutane 1,1-diacetate (**10b**) (15 mg, 2 equiv, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL) as described for **1a**. TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.54. The yield was 10.8 mg (39%). ^{1}H NMR (free base): δ 8.02 (dd, J = 8.15, 0.83 Hz, 1 H, H-1), 7.91 (t, J = 8.15 Hz, 1 H, H-2), 7.54 (dd, J = 8.15, 0.83 Hz, 1 H, H-3), 6.65 (s, 1 H, H-4″), 5.52 (bs, 1 H, H-1′), 5.35 (bs, 1 H, H-7), 4.72 (s, 2 H, H-14), 4.03 (s, 3 H, 4-OCH_3), 3.97 (bs, 1 H, H-5′), 3.60 (m, 1 H, H-4′), 3.35 (d, J = 16.4 Hz, 1 H, H-10a), 3.05 (d, J = 16.4 Hz, 1 H, H-10b), 2.95 (m, 1 H, H-3′), 2.61 (m, 2 H, H-1″), 2.40 (m, 1 H, H-8a), 2.26 (m, 1 H, H-8b), 2.01 (s, 6 H, 2 × $OCOCH_3$), 1.78 (m, 1 H, H-2′a), 1.73 (m, 1 H, H-2′b), 1.55 (s, 6 H, 2 × $OCOCH_3$), 1.52 (m, 2 H, H-2″), 1.35 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 744 (M + H)$^+$. HRMS calcd for ($C_{37}H_{46}NO_{15}$) 744.2867, found 744.2889.

***N*-[[(2,2-Diacetoxyethyl)oxy]ethyl]doxorubicin Hydrochloride (1g).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), [(2,2-diacetoxyethyl)oxy]-acetaldehyde (**16**) (14.3 mg, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL), as described for **1a**. TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.6. The yield was 9.4 mg (34%). ^{1}H NMR (free base): δ 8.11 (dd, J = 8.2, 0.8 Hz, 1 H, H-1), 7.82 (t, J = 8.2 Hz, 1 H, H-2), 7.35 (dd, J = 8.2, 0.8 Hz, 1 H, H-3), 6.75 (t, 1 H, J = 5.5 Hz, H-2‴), 5.50 (t, J = 1 Hz, 1 H, H-1′), 5.35 (bs, 1 H, H-7), 4.75 (s, 2 H, H-14), 4.11 (s, 3 H, 4-OCH_3), 3.93 (m, 1 H, H-5′), 3.77 (m, 1 H, H-4′), 3.65 (m, 2 H, H-2″), 3.54 (m, 2 H, H-1‴), 3.25 (d, J = 16 Hz, 1 H, H-10a), 3.21 (m, 1 H, H-3′), 3.14 (m, 2 H, H-1″), 2.95 (d, J = 16 Hz, 1 H, H-10b), 2.35 (m, 1 H, H-8a), 2.25 (m, 1 H, H-8b), 2.24 (m, 2 H, H-2′a), 2.12 (m, 1 H, H-2′b), 2.01 (s, 6 H, 2 × $OCOCH_3$), 1.34 (d, J = 6 Hz, 3 H, H-6′). MS (FAB): m/z 732 (M + H)$^+$. HRMS calcd for ($C_{35}H_{42}NO_{16}$) 732.2504, found 732.2485.

***N*-[[(3,3-Diacetoxypropyl)oxy]ethyl]doxorubicin Hydrochloride (1h).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), [(3,3-diacetoxypropyl)oxy]acetaldehyde **(21a)** (15.2 mg, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL), as described for **1a**. TLC: silica gel, $CHCl_3-MeOH$, 10:1; R_f = 0.6. The yield was 9.4 mg (34%). ^{1}H NMR

(free base): δ 8.10 (dd, *J* = 8.1, 0.8 Hz, 1 H, H-1), 7.82 (t, *J* = 8.1 Hz, 1 H, H-2), 7.34 (dd, *J* = 8.1, 0.8 Hz, 1 H, H-3), 6.73 (t, *J* = 5.4 Hz, 1 H, H-3‴), 5.52 (t, *J* = 1 Hz, 1 H, H-1′), 5.4 (bs, 1 H, H-7), 4.73 (s, 2 H, H-14), 4.12 (s, 3 H, 4-OCH_3), 3.96 (m, 1 H, H-5′), 3.75 (m, 1 H, H-4′), 3.62 (m, 2 H, H-2″), 3.53 (m, 2 H, H-1‴), 3.25 (d, *J* = 16 Hz, 1 H, H-10a), 3.20 (m, 1 H, H-3′), 3.15 (m, 2 H, H-1″), 2.95 (d, *J* = 16 Hz, 1 H, H-10b), 2.38 (m, 1 H, H-8a), 2.35 (m, 1 H, H-8b), 2.24 (m, 1 H, H-2′a), 2.15 (m, 1 H, H-2′b), 2.02 (s, 6 H, 2 × $OCOCH_3$), 1.95 (tt, 2 H, *J* = 5.4, 2 Hz, H-2‴), 1.35 (d, *J* = 6 Hz, 3 H, H-6′). MS (FAB): *m/z* 746 $(M + H)^+$.

***N*-[[(3,3-Diacetoxypropyl)oxy]prop-1-yl]doxorubicin Hydrochloride (1i).** The compound was prepared from doxorubicin hydrochloride (20 mg, 0.035 mmol), [(3,3-diacetoxypropyl)oxy]propionaldehyde (**21b**) (16.3 mg, 0.07 mmol), and $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv, 0.024 mmol) in CH_3CN-H_2O (2:1) (5 mL) as described for **1a**. The yield was 10.25 mg (38%). ¹H NMR (free base): δ 8.07 (dd, *J* = 8.1, 0.8 Hz, 1 H, H-1), 7.80 (t, *J* = 8.1 Hz, 1 H, H-2), 7.37 (dd, *J* = 8.1, 0.8 Hz, 1 H, H-3), 6.74 (t, *J* = 5.4 Hz, 1 H, H-3‴), 5.60 (t, *J* = 1 Hz, 1 H, H-1′), 5.32 (bs, 1 H, H-7), 4.75 (s, 2 H, H-14), 4.14 (s, 3 H, 4-OCH_3), 4.01 (m, 1 H, H-5′), 3.65 (t, 2 H, H-3″), 3.51 (m, 1 H, H-4′), 3.45 (m, 2 H, H-1‴), 3.27 (d, *J* = 16 Hz, 1 H, H-10a), 3.23 (m, 1 H, H-3′), 2.99 (d, *J* = 16 Hz, 1 H, H-10b), 2.25 (m, 2 H, H-8a, H-8b), 2.08 (s, 6 H, 2 × $OCOCH_3$), 2.05 (m, 2 H, H-2‴), 2.00 (m, 2 H, H-2′a, H-2′b), 1.35 (d, *J* = 6 Hz, 3 H, H-6′). MS (FAB): *m/z* 760 $(M + H)^+$. HRMS calcd for $(C_{37}H_{46}NO_{16})$ 760.2817, found 760.2814.

***tert*-Butyl *N*-(4-Carboxybut-1-yl)doxorubicin Hydrochloride (25).** The compound was prepared from doxorubicin hydrochloride (25 mg, 0.043 mmol), *tert*-butyl 4-oxobutanoate **(24)** (16.6 mg, 0.11 mmol), and $NaBH_3CN$ (30 μL, 0.67 equiv, 0.030 mmol) in CH_3CN-H_2O (2:1) (5 mL) as described for **1a**. The product was purified by chromatography on a thick layer of silica gel. The yield was 18.1 mg (58%). ¹H NMR (free base): δ 8.02 (d, *J* = 8 Hz, 1 H, H-1), 7.79 (t, *J* = 8 Hz, 1 H, H-2), 7.40 (d, *J* = 8 Hz, 1 H, H-3), 5.51 (d, 1 H, H-1′), 5.31 (bs, 1 H, H-7), 4.77 (s, 2 H, H-14), 4.09 (s, 3 H, 4-OCH_3), 3.99 (q, 1-H, H-5′), 3.64 (m, 1 H, H-4′), 3.25 (d, *J* = 19 Hz, 1 H, H-10a), 2.98 (d, *J* = 19 Hz, 1 H, H-10b), 2.86 (m, 1 H, H-3′), 2.65 (t, *J* = 7 Hz, 2 H, H-3″), 2.38 (d, *J* = 15 Hz, 1 H, H-8a), 2.24 (t, *J* = 7 Hz, 2 H, H-1″), 2.16 (dd, *J* = 15, 4 Hz, 1 H, H-8b), 1.75 (m, 4 H, H-2′a, H-2′b, H-2″), 1.41 (s, 9 H, $C(CH_3)_3$), 1.38 (d, *J* = 7 Hz, 3 H, H-6′). MS (FAB): *m/z* 686 $(M + H)^+$ $(C_{35}H_{44}NO_{13})$.

***N*-(4-Carboxybut-1-yl)doxorubicin Hydrochloride (1l).** *tert*-Butyl *N*-(4-carboxybut-1-yl)doxorubicin hydrochloride **(25)** (10 mg, 0.0139 mmol) was dissolved in CH_2Cl_2 (5 mL). Trifluoroacetic acid (0.5 mL) was added, and the mixture was allowed to stand for 1 h at room temperature. It was then evaporated to dryness, and the residual solid was purified by chromatography on a thick layer of silica gel using $CHCl_3$-MeOH (8:2) as eluent. The product (7.3 mg) was dissolved in $CHCl_3-CH_3CN$ (1:1), 20 μL of a 1 M solution of HCl in ether was added, and the solution was evaporated to give **11**. MS (FAB): *m/z* 630 $(M + H)^+$ $(C_{31}H_{36}NO_{13})$.

Stability Studies. The stability of the compounds in the presence of the presence of porcine liver carboxylate esterase was determined as previously described.[2] The specific activity of the preparation (Sigma Chemical Co., St. Louis, MO) was 200 units/mg of protein, where 1 unit is defined as the amount that will hydrolyze 1.0 μmol of ethyl butyrate to butyric acid and ethanol per minute at pH 8.0 and 25 °C.

Growth Inhibition Studies. The CHO/DOX cells used in this study were originally developed by Sen et al.[8] The cells express elevated levels of P-glycoprotein and are cross-resistant with vincristine and vinblastine. The Ca 755 cells and the Panc 02 cells were obtained from the National Cancer Institute. To test for drug growth inhibitory effects, cells in exponential growth at an initial density of 1.5×10^4 cells/mL were incubated in McCoy's 5A media containing 10% FCS (total final volume, 2 mL), with various drug concentrations over the range 2×10^{-5} to 2×10^{-12} M for 6 days at 37 °C in 12-well plates. The cells were fixed with 10% formaldehyde solution (500 μL/well) for 10 min and then stained with a solution of 0.1% crystal violet (250 μL/well) for 20 min. The medium was decanted, and the plates were gently rinsed with water and air-dried. The cells were counted under an inverted phase-contrast microscope. The surviving fractions were calculated, dose−response curves constructed, and IC_{50} values (the drug concentrations that inhibited colony formation by 50%) determined. Each value represents the average of duplicate determinations.

Acknowledgment. The excellent technical skills of B. F. Pan, M.S., in the cell growth inhibitory studies is gratefully acknowledged. This work was supported by NIH Grant CA 71527.

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JM9706980