sample, however, had mp 231–233 °C dec. HPLC (elution solvent 9:1 H_2O–acetonitrile): the retention time of **3a** varied between 7.5 and 9 min, with adenosine varying between 5.5 and 7 min. For a given run, the ratio of retention times was close to 1.3 (8-NH_2-6-F-PuR/Ado). The stability of **3a** in buffered solution at pH 4, 7, and 10 was monitored by HPLC. At pH 7 and 10, **3a** was stable indefinitely at room temperature, while at pH 4 **3a** was slowly converted to 8-aminoinosine: ^{1}H NMR (100 MHz, Me_2SO-d_6) δ 3.66 (m, 2, 2 H-5′), 4.01 (m, 1, H-4′), 4.16 (m, 1, H-3′), 5.20, 5.37, 5.63 (3 br s, 3, 2′,3′,5′-OH), 6.03 (d, 1, $J_{1'2'}$ = 7 Hz, H-1′), 7.48 (br s, 2, NH_2), 4.67 (m, 1, H-2′), 8.25 (s, 1, H-2). Addition of D_2O caused the disappearance of all exchangeable protons and also allowed assignment of $J_{2'3'}$ = 5 Hz, and $J_{3'4'}$ = 2 Hz; ^{13}C NMR (100 MHz, Me_2SO-d_6) δ 61.21 (C-5′), 70.44, 70.67 (C-2′,3′), 85.80, 86.90 (C-1′,4′), 118.93 (C-5), 146.23 (C-4), 154.44 (C-6), 155.31 (C-8), 156.84 (C-2), $^1J_{C_6F}$ = 247.8 Hz, $^3J_{C_4F}$ = 13.4 Hz, $^2J_{C_5F}$ = 28.7 Hz, $^3J_{C_2F}$ = 13.3 Hz, $^4J_{C_8F}$ = 1.3 Hz; ^{19}F NMR (Me_2SO-d_6) δ 84.0 relative to $CFCl_3$ (δ 0), using $C_6H_5CF_3$ (δ −63.7) as external standard; MS (EI), m/z 285 (M^+), 254 (M − CH_2OH)$^+$, 182 (B + 30)$^+$, 153 (B + H)$^+$; UV (H_2O) λ_{max} pH 1, 263.5 nm (13.3) and 237 (5.0); pH 7, 269 (12.4), 253 (sh); pH 13, 271 (12.1), 253 (sh). Anal. Calcd for $C_{10}H_{12}FN_5O_4$: C, 42.11; H, 4.24; N, 24.55. Found: C, 42.15; H, 4.45; N, 24.52.

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N-(Cyanomethyl)- and *N*-(2-Methoxy-1-cyanoethyl)anthracyclines and Related Carboxyl Derivatives

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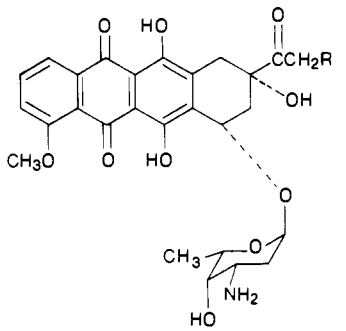
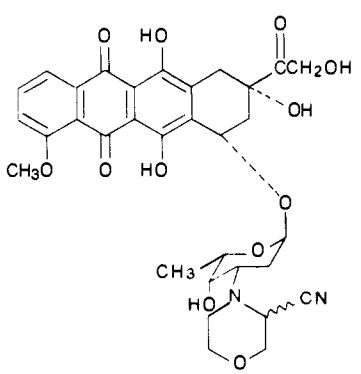
Treatment of doxorubicin with formaldehyde and NaCN afforded the *N*-(cyanomethyl) derivative as a stable α-cyano amine with but moderate antitumor activity in mice, although it was prototypal to the intensely potent α-cyanomorpholine derivative. 2-Methoxyacetaldehyde and NaCN afforded the *N*-(2-methoxy-1-cyanoethyl) derivative as an open-chain analogue of the cyanomorpholine. This analogue underwent rapid hydrolysis to doxorubicin and appeared to act as a prodrug, giving increased antitumor efficacy although with decreased potency. *N*-(Carboxymethyl)daunorubicin was a highly water-soluble but inactive analogue, synthesized by N-alkylation with ethyl iodoacetate and saponification. The similar N-alkylation of *N*-(cyanomethyl)daunorubicin demonstrated the combining of *N*-alkyl chains having different functional substituents.

3′-Deamino-3′-(3-cyano-4-morpholinyl)doxorubicin (**3**) was recently described[1-3] as the most potent of the antitumor anthracyclines, with 100- to 1000-fold decreases in dose requirement relative to the parent (doxorubicin, **2**). Several findings suggest that the added α-cyanomorpholine function in **3** is the critical feature for not only increases in potency but for apparent changes in mechanism of action. These factors include the high structural specificity for potency that is being seen in comparisons of **3** with related analogues;[4] absence in **3** of the cross resistance with **2** seen in previous analogues;[2,4] and developing evidence that **3** unlike other anthracyclines is irreversibly bound to intracellular constituents.[5-7] The α-cyano amine is a function that occurs in a few other antitumor agents such as saframycin A and cyanonaphthyridinomycin, which are postulated to bind to DNA.[8-10] An α-cyano substituent renders an amino N nonbasic. As a nonbasic analogue with intense potency, **3** is unlike other types of nonbasic anthracyclines (*N*-acyl and deamino derivatives, some with interesting antitumor efficacy in mouse screens)[11-13] that show decreased potency. Attention is thus focused on the α-CN amine of **3** as a site of interest.

We now describe the synthesis and evaluation of the *N*-cyanomethyl derivatives (**4**, **5**) as the simplest α-cyano amines from daunorubicin (**1**) and doxorubicin (**2**). The *N*-(2-methoxy-1-cyanoethyl) derivatives (**6**, **7**) are open-chain cyanomorpholine analogues derived by deletion of one ring carbon (C5″). Several *N*-(carboxyalkyl)-



1, R = H
2, R = OH



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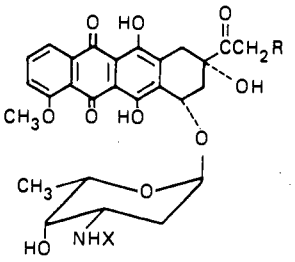
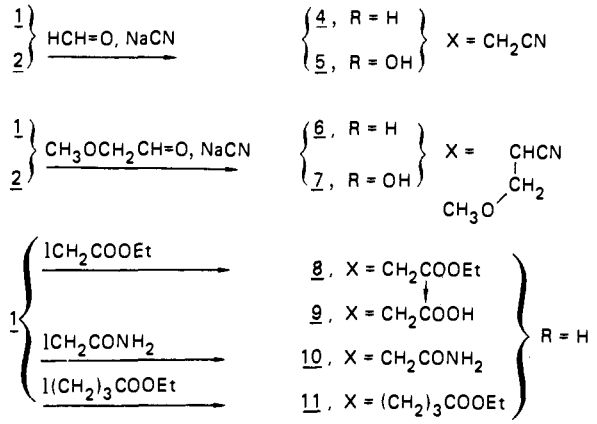
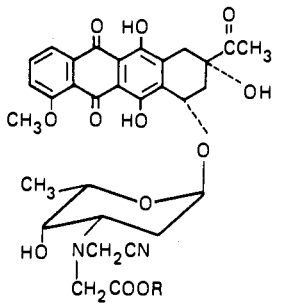
Scheme I. Target Syntheses



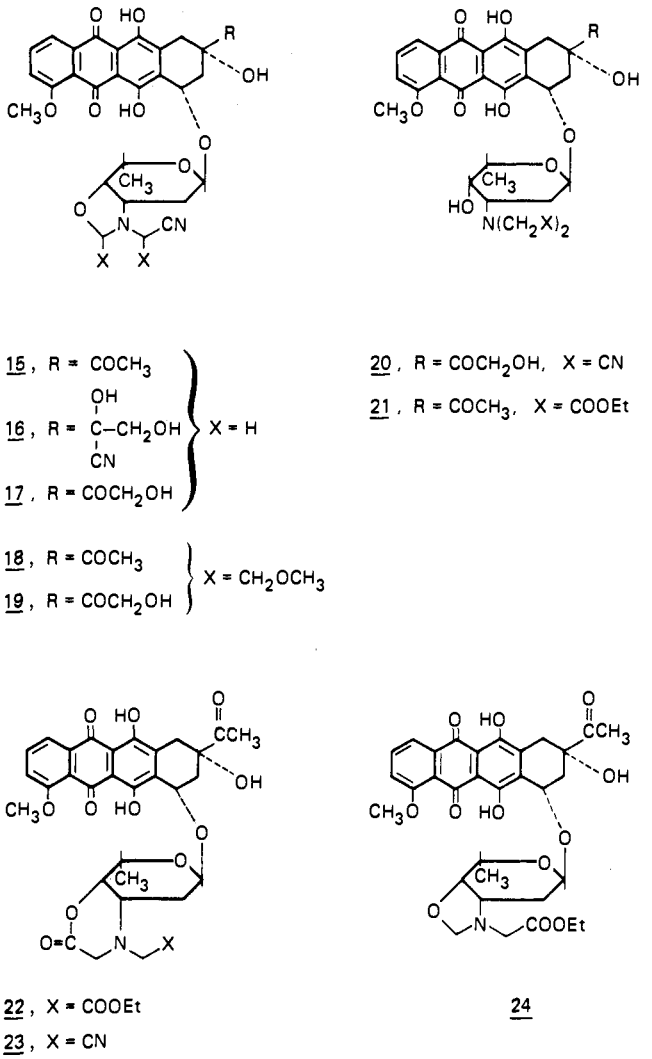




12, R = Et
13, R = Na
14, R = H

anthracyclines or their esters or amides were prepared for the first time by N-alkylation reactions. *N*-(Carboxymethyl)daunorubicin (**9**) and *N*-(carbamoylmethyl)daunorubicin (**10**) are possible hydrolysis products (in a formal sense) of **4**.

Scheme II. Intermediates and Byproducts



Synthesis. The cyanoalkyl compounds **4**–**7** were best prepared by treatment of **1** or **2** (as the hydrochlorides) with the appropriate aldehydes and sodium cyanide. A 95% yield of *N*-(cyanomethyl)daunorubicin (**4**) was obtained from **1** with formalin and NaCN. In the case of **2**, this reaction was accompanied by conversion of the more reactive 13-ketone to the cyanohydrin. A larger excess of formaldehyde–NaCN was therefore used, which caused formation of the *N*,*O*-methylene derivative, so that the major product initially was the *N*,*O*-methylene cyanohydrin **16**. Analogous bridging between the 4′-OH and the 3′-N occurred in the oxazolidino cyanomorpholino byproduct recently isolated[14] along with **3**. Treatment of **16** with acetone–chloroform and then with dilute acid (final concentration 0.05 N) afforded *N*-(cyanomethyl)doxorubicin (**5**). This product was also formed from **2** with excess iodoacetonitrile in the presence of anhydrous sodium acetate in dimethylformamide (DMF) solution, but the reaction was slow and incomplete even after 5 days. By either approach, small amounts of *N*,*N*-bis(cyano-

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Table I. Biological Screening Data

compd	C14 subst	amine subst	antitumor efficacy at opt dose vs. leukemia P388 in mice:[a] % T/C, mg/kg		inhibn of synth in leukemia L1210 cells:[b] ED_{50},[g] μM		ΔT_m of isol helical DNA in soln,[c,g] °C	log P[d] (octanol–phosphate buffer at pH 7.4)[g]
			day 1[e]	q4d 5, 9, 13[f]	DNA	RNA		
1[h]	H	NH_2		130 (8.0)	0.66	0.33	10.5	0.66
2[h]	OH	NH_2	252 (7.5)	160 (8.0)	1.6	0.58	12.8	0.07
3[h]	OH	cyanomorpholine	262 (0.012)	187 (0.075)	0.0030	0.00053	[8.7]	1.98
4	H	$NHCH_2CN$			1.8	0.82	[11.7[i]]	2.28
5	OH	$NHCH_2CN$	184 (3.1)	148 (8.0)	1.2	0.29	[13.9[i]]	1.20
6	H	$NHCH(CN)CH_2OMe$			0.51	0.51	[9.4]	2.64
7	OH	$NHCH(CN)CH_2OMe$	289 (20)		0.80	0.40	[12.5]	0.92[j]
8	H	$NHCH_2COOEt$		inact[k]	13	11	0.0	
9	H	$NHCH_2COOH$		inact[k]	>100	100	2.2	0.43
10	H	$NHCH_2CONH_2$			7.5	1.7	3.5	1.65
11	H	$NH(CH_2)_3COOEt$			1.3	0.37	8.4	2.04
12	H	$N(CH_2CN)CH_2COOEt$			7.0	3.5		
13	H	$N(CH_2CN)CH_2COONa$			14	5.3	2.2	0.03

[a] Mice injected ip with 10^6 P388 cells on day 0 and then treated ip. T/C = average survival time of treated mice/control mice = antitumor efficacy and must be ≥120% for active result. [b] ED_{50} = drug concentration for 50% inhibition of the incorporation of [^{3}H]thymidine in the DNA or [^{3}H]uridine in the RNA of actively growing L1210 cells in culture, after 3 h of exposure. Drugs were initially dissolved in Me_2SO, and the solution was diluted to a final concentration of 1% Me_2SO. [c] ΔT_m = T_m (DNA–drug complex) – T_m (DNA, calf thymus). Concentration of drug, 5.2×10^{-6} M. Concentration of DNA (P) = 5.2×10^{-5} M in 0.01 M phosphate buffer (pH 7) containing 10^{-5} M EDTA and 5% Me_2SO. Values ≤1 indicate insignificant degree of binding to DNA. [d] log P = log of ratio of concentration in organic phase/partitioned concentration in H_2O phase, measured from UV absorbances. [e] Results from Adria Laboratories. [f] Results from NCI. [g] Results from SRI. [h] Data from ref 1. [i] Biphasic curve. [j] Standard deviation for this value was ±0.3, instead of the usual ±0.03–0.05 for other compounds. [k] Inactive up to 200 mg/kg, no animal deaths.

methyl)doxorubicin (**20**) were formed (2–5% yields), showing that some reactivity at the N of **5** was retained.

The *N*-(1-cyano-2-methoxyethyl) derivatives **6** and **7** were similarly prepared, from methoxyacetaldehyde solutions freshly generated from the dimethyl acetal. The reactions were predictably slower, yields were lower, and chromatographic purification was required. Initial cyanohydrin formation at C13 was again seen with **2**. Minor byproducts were tentatively identified as the (methoxymethyl)oxazolidino derivatives **18** and **19**, although we recognized that by mass spectral analysis these structures could not be distinguished from the *N,N*-bis(1-cyano-2-methoxyethyl) compounds if the latter underwent loss of HCN and failed to show a parent peak.

Direct N-alkylation using ethyl iodoacetate, iodoacetamide, and ethyl 4-iodobutyrate was the best approach to the desired products **8**, **10**, and **11**. In general, **1** was treated with these halides in DMF in the presence of anhydrous sodium acetate. Yields after chromatographic purification were 25–40%. Minor byproducts were the diester **21** (5%) and the ester lactone **22** (1–5%). The ester function of **8** was especially labile to hydrolysis. Treatment of **8** with dilute sodium hydroxide in aqueous acetone followed by acidification of the sodium salt gave *N*-(carboxymethyl)daunorubicin (**9**). This compound may be the most water-soluble anthracycline so far encountered. Attempts at direct synthesis of **9** by alkylation with iodoacetic acid or by reductive alkylation with glyoxylic acid/$NaBH_3CN$ gave mixtures from which **9** was difficult to isolate because of the water solubility.

Direct N-alkylation of **4** could also be effected with ethyl iodoacetate, despite the nonbasicity of **4** seen in its failure to be extracted from chloroform solution with 0.1 N acetic acid. A reaction time of 4 days was required with several additions of ethyl iodoacetate and sodium acetate, and the product **12** contained about 20% of unreacted **4** by TLC analysis. Attempts to purify *N*-(cyanomethyl)-*N*-[(ethoxycarbonyl)methyl]daunorubicin (**12**) by preparative TLC gave partial conversion to the *N*-(cyanomethyl) lactone **23**. The cyanomethyl ester **12** could be converted with 1 equiv of 0.02 N NaOH in 30 min to the sodium salt **13**. This salt was stable, but when it was neutralized in aqueous acetone with dilute HCl, the resultant acid **14** underwent slow cyclization to lactone **23**. It appeared that N-alkylation of **4** might be an approach to (cyanoalkyl)anthracyclines with a second type of *N*-alkyl substituent, but less reactive iodides—iodoacetonitrile or ethyl 4-iodobutyrate—underwent little or no reaction with **4**. In an attempted alternative synthesis of **12**, treatment of **8** with formalin/NaCN gave the *N,O*-methylene derivative **24** as the major product. This was further indication of the facile formation of an oxazolidino ring involving the 3′-N and 4′-OH. It may be significant that all the examples so far are with N-substituted anthracycline derivatives, and not with 3′-NH_2.

Biological Screening

All the compounds were first screened for inhibition of DNA and RNA synthesis in leukemia L1210 cells, as measured by the concentrations to produce 50% inhibition of incorporation of [^{3}H]thymidine and [^{3}H]uridine after 3-h exposure.[15] Data are listed in Table I. It was immediately evident that the simplified α-cyano amines **4–7** lacked the potency of the cyanomorpholine **3**. These nonbasic analogues **4–7** were, however, active and comparable to **1** and **2** in this test. Most of the other analogues were considerably less active.

The doxorubicin-derived cyano amines **5** and **7** were tested against several tumor lines[16] in the mouse. Table I shows results against leukemia P388 using two dose regimens. For each compound, the optimum dose is the dose level producing the longest survival time (% T/C) of treated mice and is a measure of antitumor potency. The percent T/C is a measure of antitumor efficacy. The

(15) Test procedure from: Tong, G. L.; Lee, W. W.; Black, D. R.; Henry, D. W. *J. Med. Chem.* **1976**, *19*, 395. Modified in footnotes to Table I.

(16) Screening tests at Adria Laboratories, Inc., Columbus, OH, or under the auspices of the National Cancer Institute, Division of Cancer Treatment, Development Therapeutics Program, were done according to NCI protocols described by: Geran, R. I.; Greenberg, H. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*(2), 1–103. We thank Dr. V. L. Narayanan for the data from NCI. The editors have asked us to report that one referee objected to use of the terms potency and efficacy as defined.

Table II. Growth Inhibition in Sensitive and Resistant Cells[a]

compd	IC_{50}, μM P388/s[b]	IC_{50}, μM P388/ADR[c]	resistance index[d]
2	0.068	8.2	120
3	0.00016	0.00030	1.9
5	0.028	0.40	14
7	0.037	2.9	77

[a] After 48-h exposure to drug. Procedure as in ref 7. [b] The P388 cells routinely used in screening. [c] A P388 subline resistant to **2**, described in ref 21 and 22. [d] Resistance index = ratio of IC_{50} values for P388/ADR to P388/S.

N-cyanomethyl derivative **5** seemed twice as potent as **2** (day 1) or equipotent with **2** (days 5, 9, 13) but was poorer in antitumor efficacy in both tests. A test against leukemia L1210 showed a 4-fold increase in potency but again a small decease in efficacy for **5** (T/C 136% at 3.1 mg/kg, day 1) compared with **2** (T/C 150% at 12.5 mg/kg). The modest activity of **5** was in contrast to the impressive results from the screening of **3**. The 2-methoxy-1-cyanoethyl derivative **7** was less potent than **2** (optimum doses 20 and 7.5 mg/kg, day 1) but gave the highest T/C (289%) that we have encountered on any analogue using this regimen. A test against B16 melanoma similarly showed increased efficacy for **7** (T/C = 243% at 10 mg/kg, ip, on day 1) relative to **2** (214% at 7.5 mg/kg). Compound **7** was also active by intravenous dosing vs. B16 on days 1, 5, and 9 (T/C = 197% at the highest tested dose, 10 mg/kg), but it was inactive by oral dosing against leuekmia L1210 on days 1, 5, and 9 (highest dose, 20 mg/kg). These results suggested considerable promise for **7**. However, a preliminary stability study (described below) suggested **7** may be acting as a prodrug for **2**.

The relatively high log *P* values for **4** and **5** show the increase in lipophilicity even with these simplest α-cyano amines.

The highly water-soluble *N*-(carboxymethyl)daunorubicin (**9**) was completely inactive, both in cultured cells and in the mouse. Evidently the charged, zwitterionic character of the glycine function in **9** is a deterrent to biological action of this anthracycline. Comparison of the octanol–water partition coefficients at pH 7.4 (log *P* values, Table I) shows, on the other hand, that **9** is less polar than **2**. The corresponding ester **8** showed low potency in vitro and was also completely inactive in the mouse. This inactivity was unexpected and was attributed to the special lability of the ester and its conversion to **9**. The carboxylate **13** and ester **12** also showed decreased potency relative to **1** or **2**, despite the presence of the cyanomethyl substituent. This was similarly explained by the polarity of the carboxylate **13** and by the ready conversion of **12** to **13** (which prevented measurement of the log *P* of **12**). These compounds (**8**, **9**, **12**, **13**) were not tested in vivo. The aminobutyrate ester **11** was considerably more stable then **8** or **12** and was comparable to **2** in L1210 cells. The stable carboxamide **10** was less potent than **1** or **2** for no evident reason.

Cross Resistance. Because of the unprecedented non cross resistance[2,4] of **3**, the simplified α-cyano amines **5** and **7** were tested for this in comparison with **2** and **3**. Table II shows the growth inhibitory effects against the standard P388 cells in culture (P388/S) along with subline resistant to **2** (P388/ADR). The dose of **2** vs. P388/S had to be escalated 120 times (resistance index) to achieve 50% inhibition of P388/ADR. On the other hand, **3** was nearly equipotent in the two cell lines (resistance index only 1.9) and was thus essentially non cross resistant. Table II shows that **5** was partly cross resistant, requiring but a 14-fold dose increase; **7** was almost fully cross resistant, requiring a 77-fold increase. When tested against P388/ADR in the mouse, both **5** and **7** were fully cross resistant with **2**. No activity was observed when dosing was elevated even to toxic levels of **5** (6.25 mg/kg, ip, day 1) and **7** (15 mg/kg, ip, days 1, 5, and 9). Hence, the non cross resistance of **3** is dependent on structural features not provided by **5** and **7**.

Table III. Drug Stability over 22 h[a]

drug	pH	recovery, %
3	7	74
	4	54
	2	10
5	7	82
	4	86
	2	92
7	7	28
	4	29
	2	58

[a] Drugs dissolved in CH_3CN (**3**, **7**) or EtOH (**5**) were diluted 1:1 with phosphate buffer (pH 7), citrate buffer (pH 4), and 0.1 N HCl–KCl (pH 2). The solutions were stored at 23 °C for 22 h and 0.010-mL aliquots were analyzed by reversed-phase HPLC on a Nova C_{18} 5-μm column in a Spectraphysics 8100LC. Elution was with 0.1 N NaH_2PO_4–CH_3CN (70:30 for **3** and **5**, 66:34 for **7**).

Stability. The chemical stability of these compounds was investigated because of the likelihood that the α-cyano amine may be a reactive function involved in their biological action. A series of α-cyano amines may be useful in attempts to correlate chemical lability with biological potency. The question of stability was raised also by consideration of the ΔT_m values[15] in Table I for helical DNA. As results accumulated, we found that the nonbasic α-cyano amines consistently gave relatively high ΔT_m values, approaching those for their basic counterparts without the CN. This was unexpected, because it is generally assumed that a basic N is required for an elevated ΔT_m, through protonation and electrostatic binding. I.e., the ΔT_m for cyanomorpholine **3** was 8.7° and for the corresponding morpholine[1] it was 9.6°. Values of 11.7 and 13.9° for the cyanomethyl compounds **4** and **5** can be compared with 15.3 and 17.5° for the corresponding *N*,*N*-dimethyl compounds[17] (in the absence of monomethyl derivatives). However, Table I, footnote *i*, indicates that the ΔT_m determinations for **4** and **5** gave anomalous biphasic curves. One explanation for this might be a chemical change during heating of the drug–DNA solution such as the DNA alkylation proposed[8-10] for other types of cyano amines. Biphasic curves were not observed for **3**, **6**, or **7**, but in these cases a change perhaps occurred at an earlier stage of heating in the determination.

Results of a preliminary stability study are shown in Table III. As a standard for comparison, **3** showed reasonable stability at pH 7 with 74% remaining after 22 h, but at pH 2 there was almost complete loss. Most of the products were not eluted from HPLC. The only identified product at pHs 7 and 4 was *N*-(2-hydroxyethyl)daunorubicin;[18] at pH 2 there was additionally hydrolytic cleavage to aglycon, as expected. With **5** there was very little decomposition, and the only identified product was **2** with the expected aglycon at pH 2. With **7** the results were quite different. There was extensive hydrolysis of **7**, forming **2** as the only product at pHs 7 and 4 and with no

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losses; **2** was accompanied by some aglycon at pH 2. For both **5** and **7**, unlike **3**, there was less decomposition at pH 2, suggesting there may be some degree of N protonation of **5** and **7** that is protective against hydrolysis.[19,20] Compared with **3**, the differences in pH dependency and in the products formed show that **5** and **7** break down by a different mechanism. The facile hydrolysis of **7** at neutral pH shows that the biological effects of **7** must be largely explained by the slow release of **2**. The facile hydrolysis of **7** is also seen in the log *P* determination (Table I, footnote *j*) where exceptionally large variations were observed.

Conclusions. This study demonstrates the direct synthesis of simple α-cyano amines in the anthracycline series. The new analogues were active against tumors but lacked the intense potency of the cyanomorpholine **3** and the activity of **3** against doxorubicin-resistant tumors. Those biological properties of **3** therefore depend on chemical and structural properties of **3** that are not provided by **5** and **7**. These α-cyano amines vary widely in chemical reactivity—both in rate and in mechanism—as measured by hydrolytic stability. Hence, they may reasonably differ in biological effects.

The synthesis of **13** combining α-cyanomethyl and α-carboxymethyl substitution of N demonstrates further possibilities for combining other functions with cyano amine. Synthesis of **9** bearing a zwitterionic glycine side chain demonstrated two effects of this polar unit: greatly increased water solubility and complete loss of biological action.

Experimental Section

Solutions in organic solvents were dried over anhydrous Na_2SO_4 and filtered through Celite. Evaporations were carried out under reduced pressure (bath ≤35 °C) on a rotary evaporator. Residues from CH_2Cl_2 or $CHCl_3$ solutions were generally solvated glasses. Evaporating solutions of these residues in CH_2Cl_2–CH_3OH mixtures (4:1 and then 1:2) gave amorphous solids that could be triturated with CH_3OH and dried free of solvation. Thin-layer chromatography (TLC) was carried out on silica gel (0.25 mm GF; Analtech) plates. Preparative TLC was done on 20 × 20 cm plates with 0.5, 1.0, and 2.0 mm of silica gel. Product purity was quantified by reversed-phase analytical HPLC, monitoring at 254 nm, flow rate 2 mL/min. System A was a Waters RCM-100 Radial Compression Separation System using a 10-μm, Radial Pak C-18 column in 0.05 M citrate buffer (pH 4.0) and CH_3CN (55:45). System B was a Specra-Physica SP-8100LC using a Waters Z-module Radial-Pak Nova C-18 5-μm column in 0.1 M NaH_2PO_4 (pH 4.4) and CH_3CN and is now preferred for better resolution and reproducibility (ratio of buffer to solvent given for each compound). UV-Vis spectra (Table IV, supplementary material) were routinely determined on a Perkin-Elmer Model 575 recording spectrometer and showed no changes in chromophore. ^1H NMR spectra in $CDCl_3$ were routinely determined with a Varian EM390 spectrometer, except as noted otherwise, and diagnostic data are given. Full details are in Table V (supplementary material). Direct chemical ionization mass spectra (DCI-MS, NDCI-MS) were determined on a Ribermag R10-10C GC–MS with NH_3 as the reagent gas.

***N*-(Cyanomethyl)daunorubicin (4).** A stirred solution of 0.564 g (1.0 mmol) of **1**·HCl in 15 mL of CH_3CN–H_2O (2:1) was treated with 0.20 mL (2.66 mmol) of 37% aqueous formaldehyde (containing 10–15% CH_3OH), and a solution of 0.130 g (2.66 mmol) of NaCN in 3.3 mL of 0.5 N HCl was added dropwise. The mixture was stirred at 23 °C in the dark for 1 h and poured into 50 mL of H_2O, and the product was extracted with two 25-mL portions of $CHCl_3$. The combined extracts were washed with 0.1 N HOAc (3 × 25 mL), H_2O (25 mL), and 5% aqueous $NaHCO_3$ (25 mL), dried, and evaporated. The residue was dissolved in 10 mL of CH_2Cl_2, diluted with 40 mL of Et_2O, and evaporated to afford 0.539 g (95%), estimated to be ≥90% pure with a few percent of **1** by analytical TLC (R_f 0.24 in $CHCl_3$–CH_3OH, 19:1) and ^1H NMR. It was identical with a sample prepared from **1** and iodoacetonitrile and purified by preparative TLC ($CHCl_3$–CH_3OH, 19:1). NMR: δ 3.56 s (NCH_2CN), 2.97 m (H3′). NDCI-MS: *m/z* 566 (M), 539 (M – HCN). DCI-MS: *m/z* 540 (M – HCN + H), 187 (sugar + H_2O), 169 (sugar), 160 (sugar + H_2O – HCN), 142 (sugar – HCN).

Weak peaks in the MS indicated the major impurity (estimated 5%) was the oxazolidino derivative **15**. NDCI-MS: *m/z* 578 (M). DCI-MS: *m/z* 199 (sugar + H_2O), 181 (sugar).

***N*-(Cyanomethyl)doxorubicin (5).** The similarly obtained product from 0.406 g (0.700 mmol) of **2**·HCl required extraction with five 25-mL portions of $CHCl_3$–CH_3OH (4:1). After they were washed with 0.1 N HOAc, the extracts yielded 0.375 g (87%) of the oxazolidino cyanohydrin **16**, estimated 90% pure with four to five minor impurities by TLC and identified by NDCI-MS [*m/z* 621 (M), 594 (M – HCN)] and DCI-MS [*m/z* 199 (sugar + H_2O), 181 (sugar)]. NMR: δ 4.68 (d), 4.22 (d, oxazolidino OCH_2N), 3.71 (s, NCH_2CN).

A solution of **7** (0.360 g) in 50 mL of $CHCl_3$–CH_3OH (19:1) was diluted with 50 mL of acetone containing 1 mg of NaCN, stirred at 23 °C for 16 h in the dark, and evaporated. Trituration of the solid residue with 5 mL of Et_2O gave 0.328 g of the crude **oxazolidino ketone 17**. A portion (35 mg) was purified by preparative TLC ($CHCl_3$–CH_3OH, 19:1) to yield 20 mg of **17**, which was 80% pure with 9% of **5**, as indicated by HPLC in system B (62:38). NDCI-MS: *m/z* 594 (M). DCI-MS: *m/z* 199 (sugar + H_2O), 181 (sugar), 172 (sugar + H_2O – HCN).

A solution of 0.282 g of **8** in 30 mL of $CHCl_3$–CH_3OH (2:1) cooled to 20 °C was treated with 1.5 mL of 1.0 N HCl and stirred at 23 °C in the dark for 17 h. It was diluted with 50 mL of $CHCl_3$, washed with 5% $NaHCO_3$ (25 mL), dried, and evaporated to give 0.262 g of **5**. An additional 0.080 g of **5** was recovered from the initial HOAc wash by neutralization with aqueous $NaHCO_3$ and extraction with $CHCl_3$. A solution of the combined samples in 1.5 mL of CH_2Cl_2–CH_3OH (9:1) was applied to a 1.5 × 23 cm column of silica gel in CH_2Cl_2–CH_3OH (99:1). The column was eluted with CH_2Cl_2–CH_3OH (99:1, 200 mL; 98:2, 100 mL; 97:3, 100 mL; 96:4, 100 mL; 95:5, 200 mL; 90:10, 100 mL). Fractions A (300 mL), B (95 mL), and C (75 mL) were set aside. Fraction D (120 mL) was evaporated, and the residue was triturated with Et_2O to give 0.237 g (64% of **5**, which was 97% pure by HPLC) (B, 62:38). NMR ($CDCl_3$–CD_3OD, 19:1): δ 3.57 (s, NCH_2CN), 2.87 (m, H3′). NDCI-MS: *m/z* 582 (M), 555 (M – HCN). DCI-MS: *m/z* 187 (sugar moiety of **5** + H_2O), 169 (sugar), 160 (sugar + H_2O – HCN), 142 (sugar – HCN). Anal. ($C_{29}H_{30}N_2O_{11}$·0.5H_2O) C, H, N.

Evaporation of fraction B and trituration of the residue with Et_2O gave 22 mg (5%) of ***N*,*N*-bis(cyanomethyl)doxorubicin (20)**, which was 80% pure by HPLC (B, 62:38) with 15% of doxorubicinone. NMR ($CDCl_3$–CD_3OD, 19:1): δ 3.82 (s, NCH_2CN), 2.87 (m, H3′). NDCI-MS: *m/z* 621 (M), 594 (M – HCN), 414 (doxorubicinone). DCI-MS: *m/z* 226 (sugar moiety of **20** + H_2O), 208 (sugar).

***N*-(1-Cyano-2-methoxyethyl)doxorubicin (7).** A solution of 2.16 g (18.0 mmol) of methoxyacetaldehyde dimethyl acetal in 32.4 mL (16.2 mmol) of 0.5 N HCl was stirred and heated at 50 °C for 30 min and then cooled to 23 °C. A stirred suspension of 1.044 g (1.8 mmol) of **2**·HCl in 70 mL of CH_3CN was treated with the freshly prepared aldehyde solution and a solution of 0.882 g (18.0 mmol) of NaCN in 10 mL of H_2O. After 5 h of stirring at 23 °C in the dark, the solution was poured into 50 mL of 5% aqueous $NaHCO_3$ and extracted with $CHCl_3$ (5 × 75 mL). The combined extracts were washed with H_2O (2 × 100 mL), dried, and evaporated to give 1.12 g of crude **7** [R_f 0.25 on TLC ($CHCl_3$–CH_3OH, 9:1)], containing 10–20% of the cyanohydrin of **7**. A solution in 50 mL of $CHCl_3$–acetone (1:1) containing 1 mg of NaCN was stirred at 23 °C in the dark for 16 h, diluted with

(19) We do not suggest this demonstrates basicity adequate to explain the elevated ΔT_m values in Table I.
(20) The stability of **5** at acidic pH is inexplicably better than for some previously reported cyanomethyl compounds: Gidley, M. J.; Sanders, J. K. M. *Biochem. J.* **1982**, *203*, 331. Perhaps differences in protonation are a factor.
(21) Inaba, M.; Johnson, R. K. *Biochem. Pharamcol.* **1978**, *27*, 2123.
(22) Inaba, M.; Kobayashi, H.; Sakurai, Y.; Johnson, R. K. *Cancer Res.* **1979**, *39*, 2200.

50 mL of $CHCl_3$, washed with 5% aqueous $NaHCO_3$ (3 × 50 mL), dried, and evaporated. The residue (0.991 g) was dissolved in 10 mL of CH_2Cl_2, 50 mL of Et_2O was added dropwise with stirring, and the resultant precipitate (0.793 g was collected). An additional 0.054 g was obtained from the mother liquors. A solution of the combined product in 5 mL of CH_2Cl_2 was applied to a 1.7 × 38.5 cm column of silica gel in CH_2Cl_2. The column was eluted with CH_2Cl_2 (150 mL) and then CH_2Cl_2–CH_3OH (99:1, 450 mL; 98:2, 1000 mL; 97:3, 100 mL; 95:5, 200 mL; 80:20, 200 mL). Eluate fraction A (1020 mL) was set aside. Fraction B (350 mL) was evaporated and the residue triturated with 15 mL of Et_2O to yield 0.517 g (45%) of **7**, which was 94% pure by HPLC (B, 69:31). The presence of two diastereoisomers was indicated in the ^1H NMR at 300 MHz by the presence of two triplets (J = 4 Hz) for NCHCN at δ 3.72 and 3.66 and two singlets for OMe at δ 3.39 (s), 3.38 (s), δ 3.04 (m, H3′). NDCI-MS: m/z 626 (M), 599 (M – HCN). DCI-MS: m/z 600 (M – HCN + H), 231 (sugar + H_2O), 213 (sugar), 204 (sugar – HCN + H_2O), 186 (sugar – HCN). Weak peaks at m/z 655 (NDCI) and 260 and 242 (DCI) were attributed to fragmentation of the (methoxymethyl)oxazolidino derivative **19** as impurity. Anal. ($C_{31}H_{34}N_2O_{12}$·0.5H_2O) C, H, N.

***N*-(1-Cyano-2-methoxyethyl)daunorubicin (6).** Reaction of 1·HCl (0.056 g) with methoxyacetaldehyde and NaCN according to the procedure for **7** afforded after chromatography 0.012 g (20%) of **6**. Purity of 96% and a diastereoisomeric ratio of 52:48 was disclosed by HPLC (B, 63:37). NMR: δ 3.72 (m, NCHCN), 3.38 (s), 3.36 (s, OMe of diastereoisomers), 3.02 (m, H3′). NDCI-MS: m/z 610 (M), 583 (M – HCN). DCI-MS: m/z 584 (M – HCN + H), 231 (sugar moiety of **6** + H_2O), 213 (sugar), 204 (sugar + H_2O – HCN), 186 (sugar – HCN). Weak peaks at m/z 639 (NDCI) and 640 (DCI) were attributed to the (methoxymethyl)oxazolidino derivative **18** as impurity. Weak peaks at m/z 527 (NDCI) and 528 (DCI) were assigned to **1** as impurity.

***N*-[(Ethoxycarbonyl)methyl]daunorubicin (8).** A mixture of 1.69 g (3.00 mmol) of **1**·HCl, 0.37 mL (3.0 mmol) of ethyl iodoacetate, and 0.492 g (6.00 mmol) of anhydrous NaOAc in 30 mL of dry dimethylformamide (DMF) was stirred in the dark at 23 °C for 24 h and then evaporated. The residue was partitioned between $CHCl_3$ and 5% aqueous $NaHCO_3$. The $CHCl_3$ solution (130 mL) was extracted with 0.1 N HOAc (5 × 25 mL) and evaporated to yield 1.13 g of product. The acidic extracts were basified with $NaHCO_3$ and extracted with $CHCl_3$ to give 0.741 g of unreacted **1**. A solution of the product in 5 mL of CH_2Cl_2 was filtered, concentrated to 2 mL, and applied to a column (2.2 × 35 cm) of silica gel in CH_2Cl_2. The column was eluted with CH_2Cl_2 (200 mL) and CH_2Cl_2–CH_3OH (99:1, 400 mL; 98:2, 400 mL; 97:3, 400 mL; 90:10, 300 mL). An initial fraction (1210 mL) was set aside (partial workup and mass spectral analyses indicated it contained dialkylation product **21** and lactone **22**). The next 90 mL of eluate was evaporated to yield 482 mg (26%) of ester (93% **8** with 4% of methyl ester, by NMR; presumably the transesterification would be avoided by use of EtOH in the eluent. A portion (300 mg) was crystallized from 5 mL of absolute EtOH to afford 288 mg of fibrous needles: mp 99–103 °C; 97% pure with 2% of methyl ester by HPLC (A); TLC, R_f 0.24 in $CHCl_3$–CH_3OH (39:1). NMR: δ 3.38 (s, NCH_2CO_2), 4.13 (q), 1.20 (t, CO_2Et). DCI-MS: m/z 614 (M + H), 234 (sugar + H_2O), 216 (sugar). Anal. ($C_{31}H_{35}NO_{12}$) C, H, N.

***N*-(Carboxymethyl)daunorubicin (9).** A stirred solution of 0.356 g (0.580 mmol) of ester **8** in 25 mL of acetone under N_2 at 15 °C was treated with 25.0 mL of 0.1 N aqueous NaOH dropwise and stirred for 30 min at 25 °C. The solution was cooled to 0 °C, acidified with 25.0 mL of 0.1 N aqueous HCl dropwise, and concentrated to remove the acetone, and the solution at pH 3.7 was freeze-dried. The residue was dissolved in 25 mL of $CHCl_3$–CH_3OH (9:1), the solution was stored at 25 °C for 1 h and filtered to remove NaCl, and the filtrate was evaporated. A solution of the residue in 10 mL of $CHCl_3$–CH_3OH (4:1) was stirred and treated with 20 mL of CH_3CN dropwise to precipitate the acid **9**: 0.323 g (88% yield); 99% pure by HPLC (A); TLC, R_f 0.35 in $CHCl_3$–CH_3OH–H_2O (20:10:1). DCI-MS: m/z 568 (M + H – H_2O), 542 (M + H – CO_2), 206 (sugar + H_2O), 188 (sugar). Anal. ($C_{29}H_{31}NO_{12}$·2.5H_2O) C, H, N.

***N*-(Carbamoylmethyl)daunorubicin (10).** A mixture of 0.056 g (0.10 mmol) of **1**·HCl, 0.18 g (0.10 mmol) of iodoacetamide, and 0.016 g (0.20 mmol) of anhydrous NaOAc in 1.0 mL of DMF was after 48 h diluted with 5 mL of 0.1 N HOAc. The solution was washed with $CHCl_3$ (3 × 5 mL) and basified with $NaHCO_3$, and the product was extracted with $CHCl_3$ to yield 0.052 g of impure **10**. Preparative TLC ($CHCl_3$–CH_3OH, 9:1) afforded 0.023 g (37%) that was homogeneous on TLC; R_f 0.2. NDCI-MS: m/z 584 (M). DCI-MS: m/z 585 (M + H), 205 (sugar + H_2O), 187 (sugar). Anal. ($C_{29}H_{32}N_2O_{11}$·2H_2O) C, H, N.

***N*-[3-(Ethoxycarbonyl)propyl]daunorubicin (11).** By the procedure for **8**, **1**·HCl and ethyl 4-iodobutyrate (two additions) gave after 6 days a mixture of mono and dialkyl products. Preparative TLC ($CHCl_3$–absolute C_2H_5OH, 9:1) afforded a 25% yield of **11**, homogeneous on TLC ($CHCl_3$–CH_3OH, 19:1); R_f 0.20. NDCI-MS: m/z 641 (M). DCI-MS: m/z 642 (M + H), 262 (sugar + H_2O), 244 (sugar).

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Registry No. **1**·HCl, 23541-50-6; **2**·HCl, 25316-40-9; **4**, 103450-88-0; **5**, 103450-92-6; **6** (diastereomer 1), 103667-18-1; **6** (diastereomer 2), 103667-19-2; **7** (cyanohydrin), 103620-79-7; **7** (diastereomer 1), 103667-16-9; **7** (diastereomer 2), 103667-17-0; **8**, 103620-80-0; **9**, 103620-81-1; **10**, 103620-82-2; **11**, 103620-83-3; **12**, 103450-91-5; **13** (free acid), 103620-84-4; **13**, 103667-20-5; **15**, 103620-75-3; **16**, 103620-76-4; **17**, 103620-77-5; **20**, 103620-78-6; **23**, 103620-85-5; **24**, 103620-86-6; methoxyacetaldehyde dimethyl acetal, 24332-20-5; ethyl iodoacetate, 623-48-3; iodoacetamide, 144-48-9; ethyl 4-iodobutyrate, 7425-53-8.

Supplementary Material Available: Tables IV and V giving UV–vis and ^1H NMR spectral data (2 pages). Ordering information is given on any current masthead page.