Intensely Potent Morpholinyl Anthracyclines

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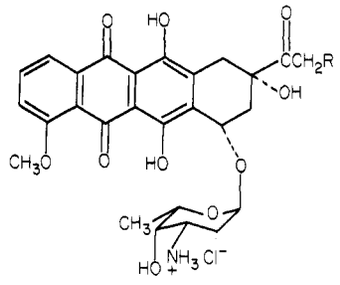
3′-Deamino-3′-(3-cyano-4-morpholinyl)doxorubicin is a new analogue that is 100 to 1000 times more potent than doxorubicin against tumors in cell culture or in mice, that is active by intraperitoneal, intravenous, or oral dosing, and that does not produce chronic myocardial lesions in mice. This analogue was encountered in studies on the reductive alkylation of doxorubicin and daunorubicin with 2,2′-oxybis[acetaldehyde], which constructs a morpholino ring incorporating the amino N. The morpholinyl nitrile byproducts are separated by virtue of their nonbasicity from the expected morpholino derivatives. The 13-dihydro and 5-imino derivatives are also described in this important new class of anthracyclines.

A useful semisynthetic approach in the search for less toxic and more active analogues of doxorubicin (**2**) is the reductive alkylation[1-4] of the sugar amino group of either **2** or daunorubicin (**1**). We recently described[3-5] the re-

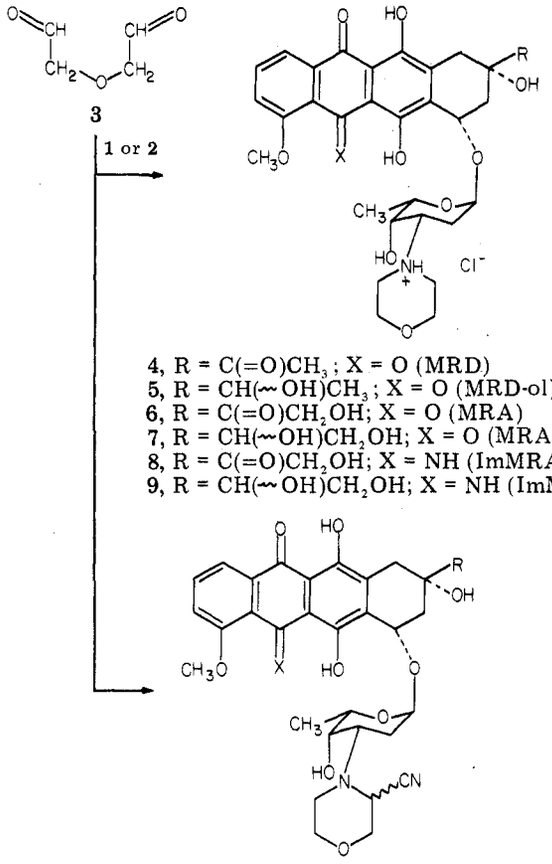


1, R = H [daunorubicin hydrochloride (D)]
2, R = OH [doxorubicin hydrochloride (A)]

ductive alkylation of **1** with 2,2′-oxybis[acetaldehyde] (**3**) and sodium cyanoborohydride, which constructs a new morpholino ring incorporating the amino N (Scheme I). The resultant morpholino derivative **4** was the most potent anthracycline then known, with a 40-fold decrease in the required antitumor dose compared to doxorubicin (**2**). Continued studies show that the morpholino derivatives constitute a family of highly potent anthracycline analogues. We now report the cyanomorpholino derivative (**12**) of **2** as a byproduct that is by far the most potent of all anthracyclines and is one of the most potent cytotoxic antitumor agents known to date.[6]

Chemistry. The first such byproduct was found in a neutral byproduct fraction[4] of **4**, the morpholino derivative of daunorubicin (**1**). Isolation of **4** involved its extraction from chloroform solution into aqueous acid with protonation of the morpholino N. The yield of **4** was only 32% (HPLC, without isolation), with 7% of the 13-dihydro derivative **5**. There was a large fraction that was not extracted into acid. This appeared to contain glycosidic substances as well as aglycons. Chromatographic separation afforded nonbasic anthracyclines that proved to be the cyanomorpholino derivative (**10**) of daunorubicin and the 13-dihydro compound **11**, largely by spectral analysis. Elemental analyses of **10** and **11** showed the presence of a second N accompanied by an additional C, compared to **4** and **5**. The UV–visible spectra (Table I; supplementary material; see paragraph at the end of paper) showed no change in the chromophore. The ^{1}H NMR spectra (Table II; supplementary material) of **10** and **11** were similar to those of **4** and **5**. At 360 MHz, **10** showed doubled resonance patterns for many of the protons compared to **4**. In addition, **10** could be resolved by analytical HPLC into two

Scheme I



4, R = C(=O)CH$_3$; X = O (MRD)
5, R = CH(~OH)CH$_3$; X = O (MRD-ol)
6, R = C(=O)CH$_2$OH; X = O (MRA)
7, R = CH(~OH)CH$_2$OH; X = O (MRA-ol)
8, R = C(=O)CH$_2$OH; X = NH (ImMRA)
9, R = CH(~OH)CH$_2$OH; X = NH (ImMRA-ol)

10, R = C(=O)CH$_3$; X = O (MRD-CN)
11, R = CH(~OH)CH$_3$; X = O (MRD-CN-ol)
12, R = C(=O)CH$_2$OH; X = O (MRA-CN)
13, R = CH(~OH)CH$_2$OH; X = O (MRA-CN-ol)
14, R = C(=O)CH$_2$OH; X = NH (ImMRA-CN)
15, R = CH(~OH)CH$_2$OH; X = NH (ImMRA-CN-ol)

peaks. This was as expected for **10**, from the lack of chiral control in the attachment of CN and the consequent ex-

† SRI.
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(2) Acton, E. M. In "Anthracyclines: Current Status and New Developments", Crooke, S. T.; Reich, S. D., Eds.; Academic Press: New York, 1980; Chapter 3.
(3) (a) Acton, E. M.; Mosher, C. W.; Gruber, J. M. "Abstracts of Papers"; 182nd National Meeting of the American Chemical Society, New York, NY, Aug 23–28, 1981; American Chemical Society: Washington, DC, 1981; Abstr CARB 10. (b) Acton, E. M.; Mosher, C. W.; Gruber, J. M. In "Anthracycline Antibiotics"; El Khadem, H. S., Ed.; Academic Press: New York, 1982; p 119.
(4) Mosher, C. W.; Wu, H. Y.; Fujiwara, A. N.; Acton, E. M. *J. Med. Chem.* **1982**, *25*, 18.

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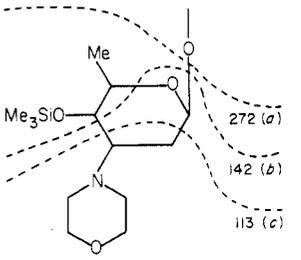
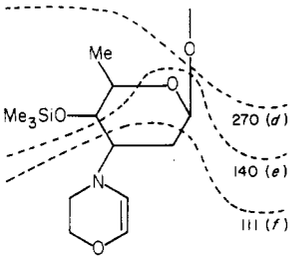
Chart I. Mass Spectral Fragments of the Sugar

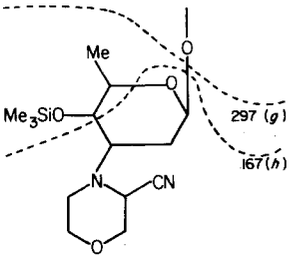
From Electron Impact Spectroscopy (EI-MS) of Silylated Compound

silylation at 80 °C

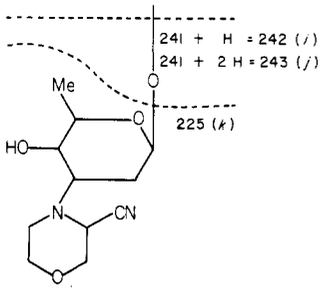


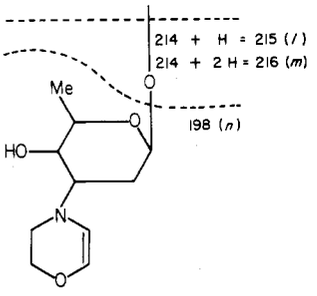


silylation at room temperature



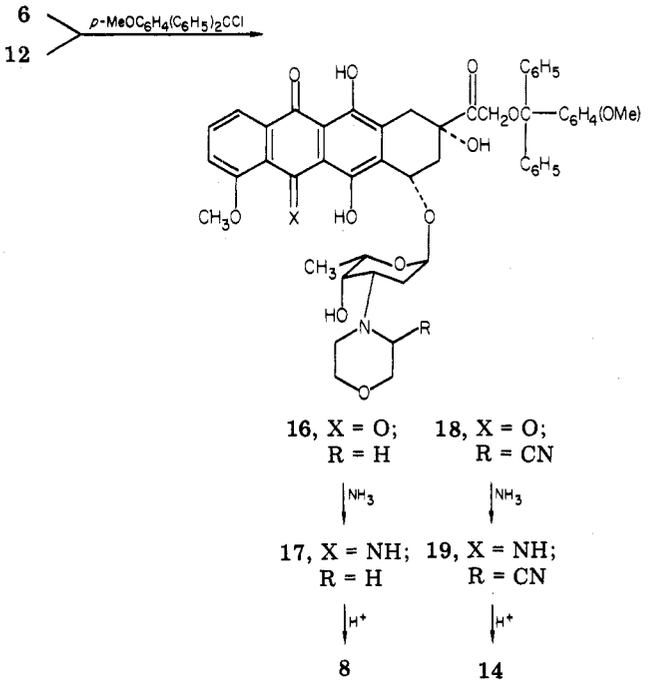
From NH_3 Desorption Chemical Ionization Spectroscopy (DCI-MS) of Underivatized Compound





istence of **10** as two diastereoisomers. Four diastereoisomers are predicted for **11**, but analytical HPLC again achieved resolution into two peaks. Initial attempts at EI-MS identification of **10** after trimethylsilylation showed only mass fragments (e.g., Chart I, *d*, *e*, *f*) after the loss of the HCN, which apparently occurred during the silylation step at 80 °C. This has been reported in the electron-impact mass spectroscopy of other cyclic α-amino nitriles.[7] Derivatization at room temperature or DCI-MS was required for observation of mass fragments (Chart I, *g*–*k*) containing CN. The actual formation of HCN, *m*/*e* 27, was observed by EI-MS of underivatized **10** heated to 130 °C. There was no sign of CN absorbance in the infrared spectrum of **10**, but CN bands are often missing in the IR of such compounds. For example, no CN absorption was found for the α-amino nitrile functionality in the natural antitumor agent saframycin A,[8] although a weak CN band was reported for the α-amino nitrile in the IR of the related cyanocycline A.[9] However, the ^{13}C NMR spectrum of **10** did show signals at δ 115.6 and 115.3 in the region expected for CN. The loss of basicity of the morpholino N was encountered not only in the failure of **10** and **11** to be extracted into aqueous acid but also in the failure to undergo the usual facile conversion to HCl salts, when **10** or **11** was isolated, redissolved in CH_2Cl_2, and treated with methanolic HCl. The absence of basicity in these α-amino nitriles is precedented in reports[10] that basicity of tertiary amines is decreased by about 6 pK units

Scheme II



by the α-CN, giving a pK_a of about 2 for N-morpholineacetonitrile.[10a] A recent study showed that saframycin A was protonated selectively at the N that is not α to the nitrile.[11] The apparent effect of the cyano substituent to suppress the basicity of the morpholino N in **10** and **11** was one of our reasons for assigning the structures with the CN adjacent to the N rather than adjacent to the morpholino O. Another reason was based upon a ^1H NMR comparison of **4** and **10** (Table II), suggesting that introduction of the CN removed two protons from the resonance assigned to CH_2NCH_2—one proton replaced and one shifted downfield—and not from the resonance assigned to

(5) Acton, E. M.; Jensen, R. A; Peters, J. H. In "Anthracycline Antibiotics in Cancer Therapy"; Muggia, F. M.; Young, C. W.; Carter, S. K., Eds.; Martinus Nijhoff: The Hague, 1982; p 205.
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(10) (a) Marxer, A. *Helv. Chim. Acta* **1954**, *37*, 166. (b) Soloway, S.; Lipschitz, A. *J. Org. Chem.* **1958**, *23*, 613. (c) Stevenson, G. W; Williamson, D. *J. Am. Chem. Soc.* **1958**, *80*, 5943.
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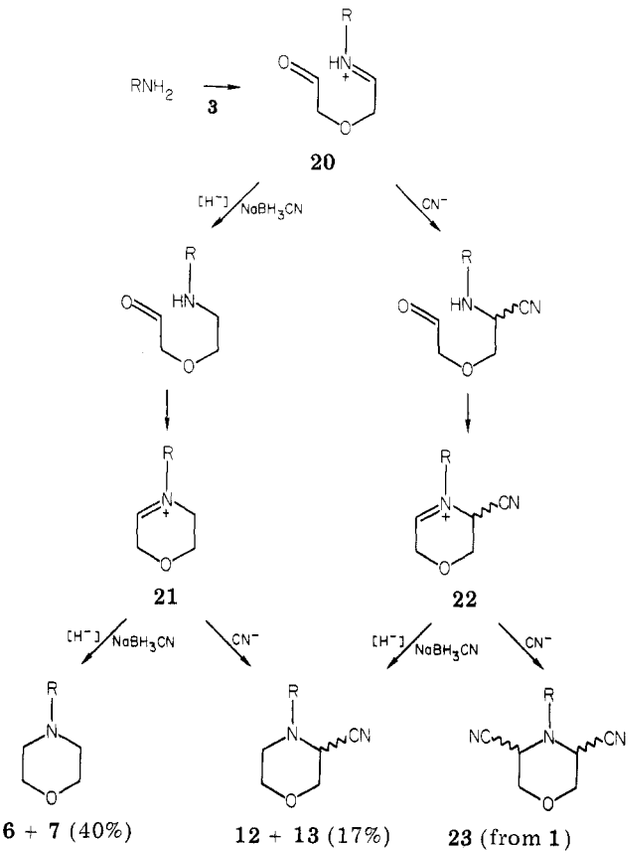
Scheme III



CH_2OCH_2. That is, in the integrated spectra, loss of two protons from the region that included CH_2NCH_2 (δ 2.65–2.35 in **4**) was concomitant with the appearance of a new one-proton multiplet (δ 3.92 in **10**) assigned to NCHCN. A similar loss of one proton and a downfield shift of a second was apparent in comparing **5** with **11**. Finally, formation of the α-cyano amine was logical on mechanistic grounds. The reductive alkylation[12] of **1** or **2** involves iminium intermediates (**20** and **21**, Scheme III) at both initial and final stages of the ring construction. Normally, hydride addition occurs at the iminium carbons of **20** and then **21**, but CN^- can intervene at either stage, and thus there are two pathways to the cyanomorpholino ring. If CN^- adds initially to **20**, it is also possible that the α-cyano iminium ion **22** will add a second CN^-. Dicyano compound **23** was, in fact, isolated in low yield from some experiments with added NaCN but no $NaBH_3CN$. The small amounts of **23** were identified from the EI-MS after trimethylsilylation at 80 °C, which showed a fragment retaining both CN substituents [*m/e* 848 [$M(Me_3Si)_3$ – Me]], as well as fragments for loss of both one [*m/e* 836 [$M(Me_3Si)_3$ – HCN], 821 [$M(Me_3Si)_3$ – HCN – Me]] and two [*m/e* 809 [$M(Me_3Si)_3$ – $(HCN)_2$], 794 ($M(Me_3Si)_3$ – $(HCN)_2$ – *Me*]] molecules of HCN. The addition of CN^- to iminium ions to form α-amino nitriles, as proposed in Scheme III, is well precedented, but we have been somewhat surprised to find only one example of this in the literature on reductive alkylation. In an N-methylation with formaldehyde and cyanoborohydride, formation of up to 25% of the *N*-cyanomethyl product was recently observed.[13] The required free CN^- may be present[13] in reagent $NaBH_3CN$, but it is also formed in the reduction, according to the proposed mechanism.[12]

The reductive alkylation was next undertaken with doxorubicin (**2**) and **3** with the deliberate intent of isolating both the morpholino and cyanomorpholino products. It appeared advantageous to conduct the reaction for a shorter time (10 min instead of 1 h as for **1**), with a lesser excess of cyanoborohydride (150% of theory instead of 300%), and to add the hydride without waiting for the dialdehyde (**3**) and amine (**2**) to form a Schiff base. As with **1**, basic and neutral products were separated by extraction and then isolated by silica gel chromatography. Purity was analyzed by reverse-phase HPLC. The major product was the morpholino derivative **6** (28% yield after conversion to the HCl salt), with 13% of the 13-dihydro compound **7**. The nonbasic cyanomorpholino derivative **12** was obtained in 14% yield, with 3% of the 13-dihydro compound **13**. These four products (58%) accounted for nearly all the material, after allowing for the losses on silica gel and other small losses in processing. There was also a few percent of an unknown, but there were essentially no aglycons. Except for **6**, these products were expected to be diastereoisomeric mixtures. Pairs of diastereoisomers produced by the CN substituent were generally resolved by HPLC but not the diastereoisomers from chirality at C-13 in the 13-dihydro compounds. Thus, two HPLC peaks were observed for **10**–**15**. This isomerism was also observable as doubled resonance patterns in the high-dispersion ^1H NMR spectra of **10**–**14**.

Synthesis of the 5-imino analogues of **6**, **7**, **12**, and **13** was undertaken to investigate possible improvements in therapeutic properties. Studies with 5-iminodaunorubicin[14-21] showed that the imino modification suppressed the redox cycling and radical-generating properties of the quinone and that the in vivo cardiotoxic potency of the drug was significantly decreased without interfering with antitumor activity. With 5-iminodoxorubicin,[22] antitumor efficacy was significantly increased, although potency decreased. A preliminary experiment with **13** showed that the cyanomorpholino function was stable during amination of the quinone with cold methanolic ammonia. Under these conditions, 13-dihydro compounds **7** and **13** were converted directly to the 5-imino derivatives **9** and **15** in 60–70% yields. The hydroxy ketone side chain of **6** and **12**, however, required intermediate protection (Scheme II) as the methoxytrityl derivatives **16** and **18**, as in the synthesis of 5-iminodoxorubicin.[22] When **18** was aminated and the methoxytrityl 5-imino derivative **19** was purified by chromatography, a partial separation of the diastereoisomers was obtained in the initial (4:1 **19a**/**19b**) and final (4:1 **19b**/**19a**) fractions. Apparently, the lipophilic character of **19** favored this separation on silica gel. The availability of **19a** and **19b** permitted deblocking to small amounts of **14a** and **14b** (again as 4:1 mixtures). The

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(19) Doroshow, J. H. *Cancer Res.* **1983**, *43*, 460.
(20) Peters, J. H.; Gordon, G. R.; Kashiwase, D.; Acton, E. M. *Cancer Res.*, in press.
(21) Jensen, R. A.; Acton, E. M.; Peters, J. H., submitted to *Cancer Res.*
(22) Acton, E. M.; Tong, G. L. *J. Med. Chem.* **1981**, *24*, 669.

Table III. Biological Screening Data

compd no.	compd abbr[a]	antitumor efficacy at opt dose vs. leukemia P388 in mice[b] d 1: % T/C (mg/kg)[k]	antitumor efficacy at opt dose vs. leukemia P388 in mice[b] q4d 5, 9, 13: % T/C (mg/kg)[l]	inhibn of synth in leukemia L1210 cells:[c,m] ED_{50}, μM DNA	inhibn of synth in leukemia L1210 cells:[c,m] ED_{50}, μM RNA	Δ T_m of isolated helical DNA in soln,[d,m] °C	log P, (octanol-phosphate buffer at pH 7.4)[e,m]
1	D		130 (8.0)	0.66	0.33	10.5	0.66
2	A	252 (7.5)	160 (8.0)	1.6	0.58	12.8	0.07
4	MRD	155 (0.25)	166 (0.20)	0.76	0.10	6.6	2.31
5	MRD-ol		132 (0.20)	2.2	0.53	4.1	2.03
10[f]	MRD-CN	175 (0.25)	197 (0.40)	0.012	0.0020	6.3	2.59
11	MRD-CN-ol		143 (0.10)	0.019	0.0020	4.2	
6	MRA	182 (0.050)	inactive[g]	0.71	0.17	9.6	1.73
7	MRA-ol	⩾145 (0.40)[h]		7.5	3.3	3.9	
12[f]	MRA-CN	262 (0.012)	187 (0.075)	0.0030	0.00053	8.7	1.98
13	MRA-CN-ol	183 (0.050)	150 (0.20)	0.021	0.0030	4.5	1.56
8	ImMRA	⩾215 (4.0)[i]	inactive[j]	2.6	1.3	4.3	1.80
9	ImMRA-ol		⩾161 (50)[h]	>100	24	1.6	1.43
14[f]	ImMRA-CN	⩾165 (0.8)[h]	157 (0.50)	0.031	0.0047	3.2	1.97
15	ImMRA-CN-ol			0.58	0.075	0.8	

[a] Abbreviations: A, doxorubicin; D, daunorubicin; ol, 13-dihydro; MR, morpholino; CN, cyano; Im, 5-imino. [b] 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. [c] 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. Drugs were initially dissolved in Me_2SO, and the solution was diluted to a final concentration of 1% Me_2SO. [d] ΔT_m = T_m of DNA-drug complex − T_m of 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} EDTA and 5% Me_2SO. Values ⩽1 indicate insignificant degree of binding to DNA. [e] Log P = log of ratio of concentration in organic phase/partitioned concentration in H_2O phase, measured from UV absorbances. [f] Mixture of diastereoisomers measured by HPLC: **10** = 55:45; **12** = 60:40; **14** = 70:30. [g] Inactive up to 0.5 mg/kg top dose; average animal weight loss = 7 g, but no deaths. [h] Incomplete; require test at higher doses. [i] Incomplete; require test at lower doses. [j] Inactive up to 3 mg/kg; animal deaths at 6 mg/kg. [k] Results from Adria Laboratories. [l] Results from NCI. [m] Results from SRI. We thank Dorris L. Taylor for these results.

amounts were sufficient for in vitro screening to test for the biological importance of the diastereoisomerism. The less lipophilic **14a** and **14b** could be resolved by HPLC but not by thin-layer chromatography on silica gel. The detritylations of **19a** and **19b** under the usual conditions in 90% acetic acid at room temperature gave yields (45–50%) that suggested losses due to decomposition. Consequently, the main fraction of **19** was treated with cold 50% trifluoroacetic acid for 2 min for cleavage to **14** in 84% yield.

Biological Results

Initial antitumor screening results against lymphocytic leukemia P388 in mice are compared in Table III. Two dose regimens were used for most of the compounds—injection in a single ip dose on day 1 after tumor implantation, or in three doses on days 5, 9, and 13 when the tumor had become systemic.[23] Also in Table III are test results on lymphoid leukemia L1210 cells in culture. Drug concentrations (ED_{50}) for 50% inhibition of DNA and RNA synthesis are compared, as measured by the incorporation of [^{3}H]thymidine and [^{3}H]uridine. A simple measure of drug-DNA binding is the ΔT_m, or increase in thermal denaturation temperature of helical DNA after adding the drug. Finally, the log p values give a relative measure of the lipophilicity of the analogs based on partitioning between 1-octanol and aqueous phosphate buffer at pH 7.4. This may be an important parameter. Recent studies[24,25] with **4** showed rapid uptake and intense accumulation in a human colon carcinoma cell line that were related to its increased lipophilicity.

The extraordinary potency of **12** is immediately apparent in Table III. Compared with doxorubicin (**2**) against P388 leukemia in mice, the dose requirements for **12** are decreased 600-fold (to 0.012 mg/kg; day 1) or 100-fold (to 0.075 mg/kg; days 5, 9, and 13). At the same time, antitumor efficacy appears to be somewhat improved, with a significant increase in T/C at least in the test on days 5, 9, 13 (from 160% for **2** to 187% for **12**). The potency increase was predicted in L1210 cells, where the ED_{50} for **12** was decreased 500-fold vs. DNA and 1000-fold vs. RNA synthesis, to give ED_{50} values in the nanomolar range. Few antitumor agents of this potency in vivo or in vitro have been reported.[26,27]

It is clear from comparisons in Table III that the increased activity with **12** is related to presence of the morpholino ring bearing the cyano substituent. We previously found[4] 40-fold increased potency in going from daunorubicin (**1**) to the morpholino derivative **4** (opt dose = 0.2 mg/kg). The corresponding cyanomorpholino derivative **10** was active at comparable doses in the mouse (either higher or lower T/C, depending on regimen); potency increases from **4** to **10** were seen only in L1210 cells. In the doxorubicin series, antitumor potency in the mouse was increased 150-fold from **2** to the morpholino derivative **6** with a single dose on day 1. Interestingly, in the delayed,

(23) 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.; Abbot, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3* (2), 1–103. We thank Dr. V. L. Narayanan for the data from NCI.

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(26) Smith, C. R.; Weisleder, D.; Matsumoto, G.; Clardy, J. "Book of Abstracts", 185th National Meeting of the American Chemical Society, Seattle, Mar 20–25, 1983; American Chemical Society: Washington DC, 1983; Abstr MEDI 74. Sesbanimide showed T/C = 170% at 0.01 mg/kg vs. P388.

(27) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. *J. Antibiot.* **1981**, *34*, 1119. CC-1065 showed T/C = 160–210% at 0.02–0.20 mg/kg vs. P388.

Table IV. Other Tests for Antitumor Efficacy at Optimum Dose in Mice[a]

compd no.	compd abbr	leukemia P388: iv doses, d 1: % T/C (mg/kg)	leukemia P388: po doses, q4d 1, 5, 9: % T/C (mg/kg)	leukemia L1210: ip doses, d 1: % T/C (mg/kg)	B16 melanoma: ip doses, q4d 1, 5, 9: % T/C (mg/kg)
2	A	178 (10)	inactive	185 (10)	278 (10)
12	MRA-CN	152 (0.050)	⩾158 (0.10)[b]	164 (0.025)	⩾148 (0.0063)[c]

[a] See Table III, footnotes *a* and *b*. Screening at Adria Laboratories according to protocols in ref 23. [b] Incomplete; require testing at higher doses. [c] Incomplete; require testing at lower doses.

three-dose test, **6** was inactive at nontoxic doses, suggesting a loss in selectivity in this regimen. With **12**, the added CN substituent produced greater activity in each of the tests.

The 13-dihydro analogues showed somewhat lower T/C values (**5** vs. **4**; **11** vs. **10**) and sometimes lower potency (**7** vs. **6**; **13** vs. **12**).[28] The 5-imino derivatives showed lower potency (**8** vs. **6**; **14** vs. **12**). The 13-dihydro 5-imino combination required much higher doses but permitted good activity to be expressed (T/C = 161%) at those levels (50 mg/kg). The general trend was for activity to increase from the parent to the morpholino to the cyanomorpholino and to decrease with the 13-dihydro and 5-imino derivatives. This was both in mice and in cultured cells—except that in L1210 cells the striking potency increase always occurred only when the CN was added (**4** → **10**; **5** → **11**; **6** → **12**; **7** → **13**; **8** → **14**; **9** → **15**). It is tempting to seek a correlation between this apparently specific biological effect and the loss of basicity with the cyano derivatives.

The small samples of **14a** and **14b** (the largely resolved diastereoisomers of **14**) that were available were tested against L1210. The ED_{50} values were essentially identical for the two samples (0.11 and 0.11 μM vs. DNA synthesis, 0.0092 and 0.010 vs. RNA synthesis). These results from one test are not definitive, but they suggest that chirality of the CN group does not affect the activity of these structures.

The wide variations in antitumor activity seen in Table III were not reflected in the ΔT_m values. Thus, the very potent **12** (ΔT_m = 8.7 °C) showed somewhat weaker binding to DNA than **1** or **2** in this test. Both **9** and **15** (ΔT_m = 1.6, 0.8°) were essentially nonbinding to DNA, yet the ED_{50} values in L1210 cells differed widely. This was consistent with a recent finding[24] that DNA binding affinities of **2** and **4** were similar while their pharmacokinetic properties were strikingly different.

The log *P* measurements showed that the morpholino analogues as a class are much more lipophilic than the parent drugs **1** and **2**, to a degree that might not have been predicted, apart from the recent study[24] with **4**. It seemed logical, however, that the log *P* was always increased with the CN substituent, was increased from the doxorubicin to the daunorubicin analogue, was decreased with the 13-dihydro derivative, and was little changed with the 5-imino derivative. Generally, the differences in log *P* between compounds were inadequate to explain the differences in activity between compounds. The log *P* values in Table III, however, were measured at pH 7.4 and do not show differences due to the complete loss of basicity with the cyano compounds. When we measured log *P* at pH 6.5, we found that the value for **6** decreased from 1.73 to 1.42, while the value for **12** remained unchanged at 1.98. This shows that the lipophilicity of **12** (and other cyano derivatives) is independent of the acidity of the system, unlike most anthracyclines. If lipophilic properties are important for drug action, the retention of lipophilicity, regardless of pH, may be a crucial factor.

Table V. Myocardial Histopathological Effects of **2** and **12**

Intravenous Dosing in Female Mice, Twice Weekly on Weeks 1, 2, 5, 6, and 7[a]

compd no.	compd abbr	dose, mg/kg	no. mice with myocardial vacuolization[b]/ no. mice examined[c]
saline control		10 mL/kg	0/11[d]
2	A	4.0	9/12[d]
12	MRA-CN	0.050	0/3[e]
		0.025	0/11[d]
		0.012	0/11[d]
		0.0063	0/10[d]

[a] Dosing schedule of Bertazzoli et al.[29,30] Surviving animals sacrificed and necropsied at week 11. [b] Tissues fixed in paraformaldehyde, embedded in glycol methacrylate, and sectioned at 2 μm, stained with Toluidine blue, and examined by light microscopy. [c] Initially 13 mice in each test group. [d] Deaths not drug related. [e] Drug-related deaths.

Additional screening tests (Table IV) showed potent activity for **12** by intravenous (iv) and oral (po) dosing against leukemia P388, by intraperitoneal (ip) dosing aginst leukemia L1210, and by ip dosing against B16 melanoma as a model solid tumor. The intense potency of **12** over **2** was retained in these tests. The increase was 200-fold against leukemia P388 (iv), 400-fold against leukemia L1210 (ip), and 1600-fold against B16 melanoma (ip). The T/C values for **12** were generally lower than for **2**, but **12** showed potent activity against P388 by the oral route, which was completely inactive for **2**. Further screening and evaluation are in progress on **12** and the other analogues (**4**–**15**). Because experimental tumors are not quantitatively predictive for human cancers, a diverse set of tests is needed in selecting an analogue for development.

An important factor in anthracycline analogue development is the possible reduction of cardiotoxicity, which is the treatment limiting side effect with **1** or **2**. Clinical anthracycline cardiotoxicity is a chronic effect, measured by morphological changes in cardiac tissue. Hence, the best validated test method[29,30] is one that requires multiple dosing in the mouse over 7 weeks, with intermediate and final intervals to allow recovery from myelosuppression, followed by light microscopy for myocardial vacuoles that resemble the clinical lesions. Results from a comparison of **2** and **12** in this test are shown in Table V. Doxorubicin (**2**) was tested at a dose level (4 mg/kg) that produced

(28) As examples accumulate, this appears to be the rule with 13-dihydro derivatives.

(29) Bertazzoli, C.; Bellini, O.; Magrini, U.; Tosana, M. G. *Cancer Treat. Rep.* **1979**, *63*, 1877.

(30) A test method based on electrocardiographic changes in the rat has been developed but less widely applied as yet. Zbinden, G.; Brändle, E. *Cancer Chemother. Rep., Part 1* **1975**, *59*, 707. Jensen, R. A.; Acton, E. M.; Peters, J. H. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 186.

antitumor activity in the mouse yet permitted the animals to survive this course of treatment. Lesions were seen in 9 of the 12 survivors. The potent analogue **12** was tested over a range of doses, starting with a low level (0.0063 mg/kg) that produced antitumor activity in some tests up to a level (0.050 mg/kg) that left only 3 survivors out of 13 test mice. There were no lesions at all with **12**, even at the highest level, which produced lethality.

These results suggest an extraordinary selectivity for **12** and this class of structures. There is intensely potent activity ip against tumors, which is retained in iv and po dosing, yet the major toxic side effect for anthracyclines appears to be absent.

Conclusions

It appears that **12** offers an extraordinary lead for new analogue development and improved chemotherapy for cancer. The anthracyclines are an exceptionally active class of structures, with access, apparently, to multiple mechanisms of action. The properties of the morpholino and cyanomorpholino subclass illustrate that with complex molecules continued analogue synthesis and evaluation can be useful even after 10–15 years. As nonbasic analogues, the cyano-substituted structures (e.g., **12**) appear to be the first with increased potency. Previous nonbasic analogues where the amino N was acylated[31] or deleted[32] were active but required high doses. Potency like that of **12** has been attained with few antitumor agents.[26,27] Apart from its potential for therapy, **12** will be a useful molecular probe of biochemical mechanisms. Studies to explain the antitumor potency without increases in DNA interactive effects or cardiotoxic effects should be of considerable importance.

Experimental Section

Solutions in organic solvents were dried over anhydrous Na_2SO_4 (use of $MgSO_4$ is incompatible with anthracycline solutions due to the formation of Mg complexes) and filtered, which was routinely done through Celite (diatomaceous earth) filter aid. 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, then 1:2) gave amorphous solids that could be triturated with CH_3OH and dried free of solvation. Column chromatography was done with 200- to 400-mesh silica gel (compound/silica gel ≃ 1:55) and monitored by TLC. Preparative TLC was done on 20 × 20 cm plates with 0.5, 1.0, or 2.0 mm of silica gel. Product purity was quantified by reverse-phase analytical HPLC on a Waters RCM-100 Radial Compression Separation System using a 10-μm, Radial Pak C-18 column in 0.05 M, pH 4.0, citrate buffer and CH_3CN or CH_3OH (organic solvent component and its ratio to the buffer given for each compound), monitoring at 254 nm, flow rate 2 mL/min. Electron-impact mass spectra (EI-MS) were determined on an LKB 9000 GC-MS at 12 eV interfaced with a PDP12 computer, generally on per(trimethylsilyl) derivatives prepared at 80 °C. Desorption chemical-ionization mass spectra (DCI-MS) were determined on a Ribermag R10-10C GC-MS with NH_3 as the reagent gas.

Reductive Alkylation of Doxorubicin (2) with 2,2′-Oxybis[acetaldehyde]. A solution of the dialdehyde was prepared by treating 6.25 g (60.0 mmol) of 1,4-anhydroerythritol[33] in 75 mL of H_2O stirred at 15–20 °C with 6.42 g (30.0 mmol) of $NaIO_4$. After 17 h at room temperature, the stirred solution was adjusted from pH 4 to 7.4 with solid $NaHCO_3$ and diluted with 75 mL of acetonitrile, with precipitation of inorganic salts. The stirred mixture was treated with a solution of 0.126 g (2.00 mmol) of $NaBH_3CN$ in 5 mL of acetonitrile–H_2O (1:1) and then with a solution of 1.16 g (2.00 mmol) of doxorubicin hydrochloride (**2**) in 30 mL of acetonitrile–H_2O (1:1). After 10 min, the reaction mixture was worked up by dilution with 50 mL of 5% aqueous $NaHCO_3$ and extraction with three 50-mL portions of $CHCl_3$. The $CHCl_3$ extracts were combined, washed with 75 mL of H_2O, and then reextracted with six 25-mL portions of 0.1 N acetic acid to remove the majority of the basic products. The majority of the neutral products remained in the $CHCl_3$.

3′-Deamino-3′-(4-morpholinyl)doxorubicin Hydrochloride (6) and 3′-Deamino-3′-(4-morpholinyl)-13-dihydrodoxorubicin Hydrochloride (7). The combined aqueous acidic extracts above were neutralized with solid $NaHCO_3$ and extracted with five 50-mL portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried and evaporated to 0.772 g of a residual glass. HPLC in citrate–CH_3CN (56:44) disclosed the presence of two major components, retention times 10.5 and 5.7 min, that proved to be the free base forms of **6** (54%) and **7** (33%). Minor amounts of the neutral products (2% of **12**; 8% of **13**) were also present. Separation was accomplished by column chromatography on silica gel, 2.2 × 33 cm. A solution of 0.98 g of the foamed glass (combined from several runs) in 3 mL of CH_2Cl_2 was added, and the column was eluted with CH_2Cl_2 (50 mL) and then CH_2Cl_2–CH_3OH (99:1, 300 mL; 98:2, 300 mL; 97:3, 900 mL; 90:10, 700 mL). Eluate fraction A (1170 mL) was set aside. Fraction B (490 mL) was evaporated to 0.444 g (30%) of residual glass, which was 99% pure as the free base of **6** by HPLC. MS (as the Me_3Si derivatives), m/e 973 [$M(Me_3Si)_5$], 901 [$M(Me_3Si)_4$], 886 [$M(Me_3Si)_4$ – Me], 871 [$M(Me_3Si)_4$ – Me_2], 812 [$M(Me_3Si)_4$ – OMe_3Si], 272 (*a*), 142 (*b*), 113 (*c*). A portion of this amorphous free base (0.042 g) was suspended in 5 mL of H_2O with stirring and dissolved by acidification to pH 4.4 with 0.6 mL of 0.1 N HCl, added dropwise. The solution was lyophilized. The light residual solid was dissolved in 0.5 mL of CH_3OH and precipitated with 5 mL of ether to yield 0.043 g (28%) of **6** as the solid hydrochloride: 99% pure; IR 3.0 (br, OH), 3.7–4.3 (NH^+), 5.82 (ketone C=O), 6.12, 6.36 (H-bonded quinone) μm. Anal. ($C_{31}H_{35}NO_{12}$·HCl·$2H_2O$) C, H, Cl, N.

Fraction C (190 mL) was set aside. Fraction D (160 mL) was then evaporated to 0.192 g of the residual free base of **7** (13%), which was 97% pure by HPLC. MS (as the Me_3Si derivatives), m/e 975 [$M(Me_3Si)_5$], 960 [$M(Me_3Si)_5$ – Me], 945 [$M(Me_3Si)_5$ – Me_2], 886 [$M(Me_3Si)_5$ – OMe_3Si], 272 (*a*), 142 (*b*), 113 (*c*). As for **6**, a 0.050-g portion of the free base was suspended in 6 mL of H_2O and dissolved by adding 0.7 mL of 0.1 N HCl to pH 4.5. The lyophilized residual solid was dissolved in 1 mL of CH_3OH and precipitated with 10 mL of ether to yield 0.051 g (12%) of **7** as the solid hydrochloride: 98% pure. Anal. ($C_{31}H_{37}NO_{12}$·1.1HCl·$2H_2O$) C, H, Cl; N: calcd, 2.02; found, 2.46.

3′-Deamino-3′-(3-cyano-4-morpholinyl)doxorubicin (12) and 3′-Deamino-3′-(3-cyano-4-morpholinyl)-13-dihydrodoxorubicin (13). The $CHCl_3$ solution that contained the neutral products from the reductive alkylation of **2** (after removal of the basic products by extraction with 0.1 N acetic acid) was dried and evaporated to yield 0.355 g of a residual glass. HPLC in citrate–CH_3CN (56:44) disclosed the presence of two components at retention times of 2.3 and 3.8 min, which proved to be **13** (4%) and **12** (82%), respectively. Another component (8.1 min) was unidentified (7%). A small amount of the basic product **6** (4%) was also found. Separation was accomplished by column chromatography on silica gel (1.5 × 35.5 cm). A solution of 0.424 g of the residual glass (combined from several runs) in 1.5 mL of CH_2Cl_2 was added, and the column was eluted with CH_2Cl_2 (50 mL) and then CH_2Cl_2–CH_3OH (99:1, 150 mL; 98:2, 150 mL; 97:3, 300 mL; 95:5, 100 mL; 90:10, 300 mL). Fraction A (445 mL) was set aside. Fraction B (80 mL) was evaporated to yield 0.217 g of **12**. This was combined with another 0.039 g of similarly obtained **12** and dissolved in 2 mL of CH_2Cl_2. The solution was diluted with 10 mL of CH_3OH and evaporated to a solid residue that could be triturated with CH_3OH (5 mL). The solid was collected on a filter and dried under vacuum to yield 0.218 g (14%) of **12**. The presence of two diastereoisomeric forms (65:35) of **12** (98% pure) was shown by HPLC in citrate–CH_3OH (40:60) and by ^1H NMR, which exhibited two distinct resonances (Table II) for many of the protons. MS (as the Me_3Si derivatives), m/e 899 [$M(Me_3Si)_4$ – HCN], 270 (*d*), 140 (*e*), 111 (*f*). Anal. ($C_{32}H_{34}N_2O_{12}$·$2H_2O$) C, H, N.

(31) For example, *N*-(Trifluoroacetyl)adriamycin 14-valerate. Israel, M.; Modest, E. J.; Frei, E. *Cancer Res.* **1975**, *35*, 1365.
(32) 3′-Deamino-3′-hydroxyadriamycin 3′,4′-diacetate. Horton, D.; Turner, W. R. *Carbohydr. Res.* **1979**, *77*, C8.
(33) Otey, F. H.; Mehltretter, C. L. *J. Org. Chem.* **1961**, *26*, 1673.

Fraction C (230 mL) was set aside. Fraction D (80 mL) was evaporated to 0.041 g (3%) of residual **13**, which was 93% pure by analytical HPLC. MS (as the Me_3Si derivatives), *m/e* 985 [$M(Me_3Si)_5$ - Me], 973 [$M(Me_3Si)_5$ - HCN], 901 [$M(Me_3Si)_4$ - HCN], 297 (*g*), 270 (*d*), 167 (*h*), 140 (*e*), 111 (*f*). Anal. ($C_{32}N_{36}N_2O_{12}\cdot 1.5H_2O$) C, H, N.

3′-Deamino-3′-(3-cyano-4-morpholinyl)daunorubicin (10) and 3′-Deamino-3′-(3-cyano-4-morpholinyl)-13-dihydrodaunorubicin (11). The similar reductive alkylation of **1** with **3** and $NaBH_3CN$ was described[4] as also yielding a neutral fraction (4.24 g from 9.2 g of **1**) which remained in $CHCl_3$ solution after the extraction with aqueous acid. In another experiment (11.7 g of **1**), the $CHCl_3$ solution after extraction with three 100-mL portions of 0.01 N HCl, 100 mL of H_2O, and 100 mL of 5% aqueous $NaHCO_3$ was dried and evaporated (0.1 mm, room temperature) to give 5.10 g of glassy residue. This residue was dissolved in CH_2Cl_2–CH_3OH (4:1), diluted with CH_3CN, and evaporated to a semisolid residue. An initial purification was accomplished by trituration of the residue with CH_3CN in successive 200- and 100-mL portions. The solids were removed by filtration. The combined filtrates were evaporated to give 2.23 g of semisolid residue. HPLC in citrate–CH_3CN (60:40) disclosed the presence of components at retention times of 5.8 and 6.4 min (23% of the total) and 12.1 and 12.8 min (36%), which ultimately proved to be **11** and **10**, respectively, as diastereoisomeric mixtures. Chromatographic purification on silica gel was accomplished by adding a solution of the semisolid in 5 mL of CH_2Cl_2 to a 3.1 × 59 cm column of silica gel in CH_2Cl_2, which was eluted with CH_2Cl_2 (500 mL) and then CH_2Cl_2–CH_3OH (99:1, 1500 mL; 98:2, 1000 mL; 97:3, 1500 mL; 90:10, 500 mL). Eluate fraction A (2550 mL) was set aside. Fraction B (190 mL) was evaporated to yield 0.48 g (3%) of **10**, 87% pure. Final purification was accomplished on a 1.1 × 27 cm column of silica gel in CH_2Cl_2. A sample (0.18 g) of **10** in 1.0 mL of CH_2Cl_2 was added, and the column was eluted with CH_2Cl_2–EtOAc (80:20, 30 mL; 60:40, 30 mL; 40:60, 30 mL; 20:80, 30 mL) and then EtOAc (175 mL). After an initial 162 mL of eluate was set aside, an 88-mL fraction was collected and evaporated to yield 0.15 g (3%) of **10**. Purity was 97% by HPLC in 60:40 citrate–CH_3CN, with a diastereoisomeric ratio of 55:45. Two resonances were seen for many of the protons in the ^1H NMR at 360 MHz. MS (Me_3Si derivatized at room temperature, 2 h) *m/e* 910 [$M(Me_3Si)_4$], 895 [$M(Me_3Si)_4$ - Me], 883 [$M(Me_3Si)_4$ - HCN], 838 [$M(Me_3Si)_3$], 823 [$M(Me_3Si)_3$ - Me], 811 [$(Me_3Si)_3$ - HCN], 739 [$M(Me_3Si)_2$, 297 (*g*), 270 (*d*), 167 (*h*), 140 (*e*), 111 (*f*). MS (underivatized) at 70 eV (130 °C in vacuo) showed a base peak at *m/e* 27 assigned to HCN. MS (NH_3–DCI) *m/e* 640 (M + NH_4), 623 (M + H), 596 (M - HCN + H), 383 (7-deoxydaunomycinone + H), 363 (dianhydrodaunomycinone + H), 243 (*j*), 216 (*m*). Anal. ($C_{32}H_{34}N_2O_{11}\cdot H_2O$) C, H, N.

Fraction C (720 mL) was set aside, and fraction D (430 mL) was evaporated to yield 0.28 g of **11**. The residue was purified by preparative TLC in $CHCl_3$–CH_3OH (19:1) to yield 0.14 g (3%) of **11** (93% pure by HPLC). MS (as the Me_3Si derivatives), *m/e* 957 [$M(Me_3Si)_5$ - HCN], 885 [$M(Me_3Si)_4$ - HCN], 813 [$M(Me_3Si)_3$ - HCN], 270 (*d*), 140 (*e*), 117 ($CH_3CHOSiMe_3$), 111 (*f*).

14-*O*-(*p*-Anisyldiphenylmethyl)-3′-deamino-3′-(4-morpholinyl)doxorubicin (16). A solution of 0.396 g (0.645 mmol) of **6** in 5 mL of dry pyridine was treated with 0.990 g (3.21 mmol) of *p*-anisylchlorodiphenylmethane and stirred in the dark at room temperature for 2 days. The solution was cooled in ice water, treated with 0.5 mL of CH_3OH, stirred for 2 h, and poured into 50 mL of 5% aqueous $NaHCO_3$ solution. The product was extracted with two 10-mL portions of CH_2Cl_2. The combined extracts were dried and evaporated to a gummy residue, which was reprecipitated from CH_2Cl_2 (2 mL) with petroleum ether (25 mL), and then from CH_2Cl_2 (8 mL) with 2:1 petroleum ether–diethyl ether (120 mL), to yield **16** (0.537 g, 94%) of ≥90% purity, according to analysis by ^1H NMR and TLC in $CHCl_3$–CH_3OH (19:1), R_f 0.57.

14-*O*-(*p*-Anisyldiphenylmethyl)-3′-deamino-3′-(4-morpholinyl)-5-iminodoxorubicin (17). A solution of 0.532 g (0.600 mmol) of **16** in 10 mL of CH_2Cl_2 was added to 30 mL of CH_3OH saturated at 0 °C with anhydrous ammonia (g). The solution was stirred at 0 °C for 1 h, stored at 3 °C for 27 h, and evaporated. The violet residue was dissolved in CH_2Cl_2–CH_3OH (4:1, then 1:2), and the solution was filtered through Celite and evaporated. This was repeated several times to remove all NH_3 and gave 0.515 g of **17**. A solution in 2 mL of CH_2Cl_2 was added to a column (1.5 × 40 cm) of silica gel in CH_2Cl_2, which was eluted with CH_2Cl_2 (50 mL) and then CH_2Cl_2–CH_3OH (99:1, 150 mL; 98:2, 150 mL; 97:3, 500 mL; 95:5, 100 mL; 93:7, 100 mL; 90:10, 200 mL). After the initial 565 mL was set aside, a 335-mL fraction was evaporated to yield 0.326 g (58%), which was homogeneous by TLC in $CHCl_3$–CH_3OH (19:1), R_f 0.32.

3′-Deamino-3′-(4-morpholinyl)-5-iminodoxorubicin Hydrochloride (8). A solution of 0.341 g (0.385 mmol) of **17** in 20 mL of 80% acetic acid was stirred in the dark for 7 h, then diluted with 50 mL of H_2O, and washed three times with 10-mL portions of $CHCl_3$. The aqueous phase was lyophilized to give 0.294 g of residual solid, which was redissolved in 40 mL of 0.1 N acetic acid. The solution was washed with three 10-mL portions of $CHCl_3$ and basified (pH 8) with solid $NaHCO_3$, and the product was extracted with three 10-mL portions of $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, dried, and evaporated. Redissolving the residue in $CHCl_3$–CH_3OH (1:10) and evaporating the solution gave 0.228 g (97% yield) of the free base of **8**: TLC (silica gel; $CHCl_3$–CH_3OH, 19:1), R_f 0.22.

A suspension of the free base in 20 mL of H_2O was stirred and treated with 3.2 mL of 0.1 N HCl dropwise while the solid gradually dissolved, maintaining the pH at or above 4.5. The solution was lyophilized, and the light solid was redissolved in 3 mL of CH_3OH, precipitated with 30 mL of ether, and collected by filtration to yield 0.228 g (87%) of **8**·HCl: 97.5% pure by HPLC in citrate–CH_3CN (20:80) with resolution from **6** (only 0.1% present); MS (as the Me_3Si derivatives) *m/e* 972 [$M(Me_3Si)_5$], 900 [$M(Me_3Si)_4$], 828 [$M(Me_3Si)_3$], 272 (*a*), 142 (*b*), 113 (*c*). Anal. ($C_{31}H_{36}N_2O_{11}\cdot 0.9HCl\cdot 2.2H_2O$) C, H, N, Cl.

3′-Deamino-3′-(4-morpholinyl)-5-imino-13-dihydrodoxorubicin Hydrochloride (9). A solution of 0.186 g (0.302 mmol) of **7** in 6 mL of CH_2Cl_2–CH_3OH (1:1) was added to 20 mL of CH_3OH that had been saturated with anhydrous NH_3 (g) at 0 °C. The solution was stored at 3 °C for 27 h and evaporated. The residue was redissolved three times in CH_2Cl_2–CH_3OH (4:1) and recovered by evaporation to completely remove ammonia and yield 0.169 g. TLC ($CHCl_3$–CH_3OH, 9:1) indicated purity was 85–90%, with only a trace of unreacted **7** (free base) but several more polar byproducts. The solid was purified by preparative TLC in $CHCl_3$–CH_3OH (9:1) to yield 0.125 g (67%, free base of **9**); a suspension in 15 mL of H_2O was gradually dissolved by treatment with 1.8 mL of 0.1 N HCl dropwise to pH 4.5. The solution was lyophilized, and the light residue was dissolved in 2 mL of CH_3OH and precipitated with 20 mL of ether to yield 0.126 g (61%) of **9**: 96% pure by HPLC (citrate–CH_3OH, 15:85); MS (of the Me_3Si derivatives), *m/e* 974 [$M(Me_3Si)_5$], 959 [$M(Me_3Si)_5$ - Me], 944 [$M(Me_3Si)_5$ - Me_2], 272 (*a*), 142 (*b*), 113 (*c*). Anal. ($C_{31}H_{38}N_2O_{11}\cdot HCl\cdot 2H_2O$) C, H, Cl, N.

14-*O*-(*p*-Anisyldiphenylmethyl)-3′-deamino-3′-(3-cyano-4-morpholinyl)doxorubicin (18). A solution of 0.241 g (0.377 mmol) of **12** in 4 mL of dry pyridine was treated with 0.587 g (1.90 mmol) of *p*-anisylchlorodiphenylmethane and stored in the dark for 2 days. The solution was cooled, treated with 0.5 mL of CH_3OH, stirred at room temperature for 3 h, and poured into 50 mL of 5% aqueous $NaHCO_3$. The product was extracted with two 10-mL portions of CH_2Cl_2, and the combined extracts were evaporated. The residue was redissolved in 3 mL of CH_2Cl_2 and precipitated by slowly adding 40 mL of ether to yield 0.268 g of **18**. Evaporation of the mother liquors and precipitation of the residue from CH_2Cl_2–ether–petroleum ether (1:40:40) gave an additional 0.065 g (total 0.333 g, 97%) of **18**. Both crops were homogeneous on TLC in $CHCl_3$–CH_3OH (39:1), R_f 0.35.

14-*O*-(*p*-Anisyldiphenylmethyl)-3′-deamino-3′-(3-cyano-4-morpholinyl)-5-iminodoxorubin (19). A solution of 0.369 g (0.405 mmol) of **18** in 8 mL of CH_2Cl_2 was added to 25 mL of CH_3OH saturated with anhydrous NH_3 (g) at 0 °C. The mixture was stored at 3 °C for 27 h and evaporated, with complete removal of NH_3, to yield 0.376 g of violet residue of 60–70% purity according to TLC in $CHCl_3$–CH_3OH (29:1). A solution in 1.5 mL of CH_2Cl_2 was added to a column (1.5 × 28 cm) of silica gel in CH_2Cl_2 and eluted with CH_2Cl_2 (50 mL) and then CH_2Cl_2–CH_3OH (99:1, 200 mL; 98:2, 300 mL; 97:3, 100 mL; 95:5, 100 mL; 90:10, 200 mL). After fraction A (360 mL) was discarded, fraction B (30 mL) was evaporated to yield 0.019 g of a 4:1 mixture of the

diastereoisomers **19a** and **19b**, as estimated by TLC in $CHCl_3$–CH_3OH (29:1) (**19a**, R_f 0.28; **19b**, R_f 0.23). Fraction C (50 mL) contained 0.103 g of **19a** and **19b** (2:3). Fraction D (45 mL) afforded 0.081 g of **19a** and **19b** (1:4).

3′-Deamino-3′-(3-cyano-4-morpholinyl)-5-iminodoxorubicin (14). A solution of 0.158 g (0.174 mmol) of **19** (combined fractions C and D) in 8 mL of ice-cold 50% trifluoroacetic acid was stirred at 0 °C for 2 min and poured into 100 mL of ice–water. The aqueous mixture was extracted with four 10-mL portions of $CHCl_3$, and the combined extracts were washed with 5% aqueous $NaHCO_3$, dried, and evaporated. The residue was redissolved in 3 mL of $CHCl_3$–CH_3OH (4:1) and precipitated by the dropwise addition of 25 mL of ether with stirring to yield 0.093 g (84%); 98% pure by HPLC in citrate–CH_3OH (40:60), which revealed a 7:3 ratio of diastereoisomers **14b**/**14a**. MS (NH_3–DCI), *m/e* 638 (M + H), 611 (M − HCN + H), 243 (*j*), 225 (*k*), 215 (*l*), 198 (*n*). Anal. ($C_{32}H_{35}N_3O_{11}\cdot H_2O$) C, H, N.

A solution of 19 mg of **19a** (from fraction B, 4:1 mixture with **19b**) in 1 mL of 90% acetic acid was stored for 7 h at room temperature and then diluted with 10 mL of H_2O and extracted with two 5-mL portions of ether to remove *p*-anisyldiphenylcarbinol. Extraction of the acidic aqueous solution with $CHCl_3$ then yielded 8 mg of diastereoisomer **14a**, 85% pure by HPLC (**14a**/**14b**, 4:1).

Similarly, **19b** (from fraction D, 4:1 mixture with **19a**) gave diastereoisomer **14b**, 86% pure (**14b**/**14a**, 4:1). The isomers could not be resolved by TLC analysis on silica gel in several solvent systems. The mass spectra of **14a** and **14b** were nearly identical with that of the above mixture (14).

3-Deamino-3′-(3-cyano-4-morpholinyl)-5-imino-13-dihydrodoxorubicin (15). A solution of 0.016 g (0.025 mmol) of **13** in 1 mL of CH_2Cl_2–CH_3OH (1:1) was added to 2 mL of CH_3OH that had been saturated with anhydrous NH_3 (g) at 0 °C. The solution was stored at 3 °C for 27 h and then evaporated, with complete removal of NH_3, to afford 0.015 g of violet residue. This residue was purified by preparative TLC in $CHCl_3$–CH_3OH (9:1) to yield 0.011 g (69%) of **15**: 92% pure by HPLC in citrate–CH_3CN (45:55); MS (as the Me_3Si derivative), *m/e* 900 [$M(Me_3Si)_4$ − HCN], 270 (*d*), 140 (*e*), 111 (*f*).

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Registry No. **1**, 20830-81-3; **2**, 23214-92-8; **3**, 7456-83-9; **4**, 80844-67-3; **5**, 79951-58-9; **6**, 89196-04-3; **6** (free base), 80790-68-7; **7**, 80787-27-5; **7** (free base), 80875-73-6; **8**, 89164-71-6; **8** (free base), 89196-05-4; **9**, 89164-72-7; **9** (free base), 89164-73-8; (α)-**10**, 89164-74-9; (β)-**10**, 89196-06-5; (α)-**11**, 89164-75-0; (β)-**11**, 89164-76-1; (α)-**12**, 89196-07-6; (β)-**12**, 89196-08-7; (α)-**13**, 89164-77-2; (β)-**13**, 89164-78-3; **14a**, 89164-79-4; **14b**, 89196-09-8; (α)-**15**, 89164-80-7; (β)-**15**, 89164-81-8; **16**, 89164-82-9; **17**, 89164-83-0; (α)-**18**, 89164-84-1; (β)-**18**, 89196-10-1; **19a**, 89164-85-2; **19b**, 89196-11-2; **23**, 89164-86-3; **24**, 89164-87-4; 1,4-anhydroerythritol, 4358-64-9.

Supplementary Material Available: UV–visible (Table I) and ^1H NMR (Table II) spectral data (3 pages). Ordering information is given on any current masthead page.

Substituted (ω-Aminoalkoxy)stilbene Derivatives as a New Class of Anticonvulsants

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A series of substituted (ω-aminoalkoxy)stilbene derivatives has been synthesized and screened for anticonvulsant activity. The effect of structural modification of these molecules on the activities has been systematically examined. Potent anticonvulsant activity was displayed by 2-[4-(4-methyl-1-piperazinyl)butoxy]stilbene (**20**) and some 2-[4-(3-alkoxy-1-piperidino)butoxy]stilbene derivatives (**21**, **37**, **38**, and **40**), as determined by maximal electroshock seizure (MES) and pentylenetetrazol-induced convulsion tests in mice. Compound **21** exhibited more potent anti-MES activity than diphenylhydantoin and carbamazepine in further pharmacological tests in rats, and its therapeutic index was superior to those of two antiepileptic drugs.

Although anticonvulsant activity is well-known to hydantoin analogues, benzodiazepine structures, and so on, there are few reports about stilbene derivatives with anticonvulsant activity.

Routine pharmacological screening in our laboratories of compounds directed toward new antiepileptic agents has shown that some substituted (ω-aminoalkoxy)stilbene derivatives antagonize the maximal electroshock seizures (anti-MES) and the pentylenetetrazol-induced convulsions (anti-PTZ) in mice. This suggested the design and synthesis of related structures in a search for more active anticonvulsant agents.

This paper describes the synthesis and primary pharmacological activities of substituted (ω-aminoalkoxy)stilbene derivatives.

Chemistry. The (4-aminobutoxy)stilbene derivatives in Tables I–IV were prepared by the ω-bromoalkylation of the corresponding 2-hydroxy-*trans*-stilbene with α,ω-dibromoalkane in the presence of KOH in *t*-BuOH and followed by amination with the corresponding amines (Scheme I). 2-Hydroxy-*trans*-stilbene has been prepared by Kauffman[1] and also might be prepared by the Wittig reaction.[2] These methods, however, give 2-hydroxy-*trans*-stilbene in a low yield. We have found that 2-hydroxy-*trans*-stilbene is obtained in a high yield by dehydration of 2-(2-phenyl-1-hydroxyethyl)phenol in DMF under reflux. The intermediate is obtained by the Grignard reaction of benzyl chloride and salicylaldehyde in THF–toluene solution (Scheme II). The substituted 2-hydroxy-*trans*-stilbene derivatives were prepared by the

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