Adriamycin Analogues. Rationale, Synthesis, and Preliminary Antitumor Evaluation of Highly Active DNA-Nonbinding *N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiester Derivatives[1,2]

Mervyn Israel,* P. Gopalakrishnan Potti, and Ramakrishnan Seshadri

Department of Pharmacology, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.
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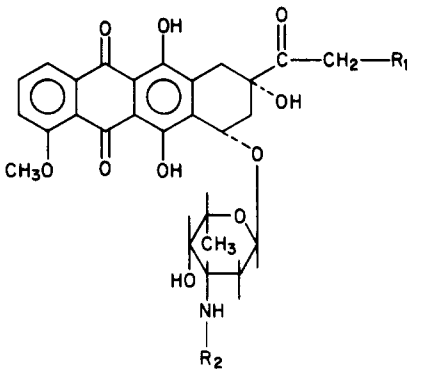
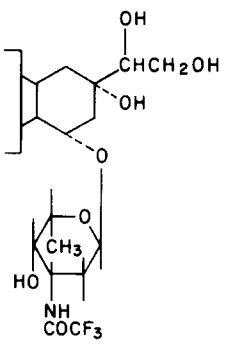
N-(Trifluoroacetyl)adriamycin 14-valerate (AD 32), a novel DNA nonbinding analogue of adriamycin with superior experimental antitumor activity, has undergone extensive clinical trial, with documentation of antitumor activity and low toxicity in human subjects. However, poor water solubility necessitates that the drug be administered to patients by continuous intravenous infusion at high dilution in a surfactant-containing formulation, with steroid prophylaxis to protect against a chest pain syndrome associated with the vehicle. On the basis of pharmacologic considerations, the title compounds have been prepared as second-generation analogues of *N*-(trifluoroacetyl)adriamycin 14-valerate with improved aqueous solubility; use is made of the available carboxylic acid function to solubilize the products in dilute aqueous alkaline medium. Target compounds were made by treating *N*-(trifluoroacetyl)-14-halodaunorubicin (bromo or iodo) with monosodium salts of dibasic acids (malonic, succinic, glutaric, adipic, pimelic, azelaic, sebacic) in aqueous acetone. All of the products showed significant in vivo antitumor activity against the murine P388 leukemia (ip tumor, ip treatment once daily on days 1, 2, 3, and 4); most compounds were superior to the +181% increase in life span afforded by adriamycin (optimal dose 3.0 mg/kg per day), one of two drugs used as positive controls for the assays. Several of the test compounds showed highly curative activity in this system, similar to *N*-(trifluoroacetyl)adriamycin 14-valerate, the other positive control agent. The hemiadipate product exhibited the most desirable properties of high antitumor efficacy (86% cure rate of all P388 tumor-bearing animals through four levels of a 40–70 mg/kg dose–response range), aqueous solubility (60 mg/mL in pH 7.4 phosphate buffer), and solution stability (no decomposition at 4 °C, 0.5% hydrolysis at 27 °C, over 24 h at pH 7.4).

With the broadest spectrum of clinical activity of any anticancer drug, the anthracycline antibiotic adriamycin (doxorubicin, **1**) continues to be of major importance in cancer medicine.[3–5] A closely related antibiotic, daunorubicin (**2**), sees clinical use primarily in the treatment of certain types of leukemias. However, many of the low growth fraction carcinomas common in older patients (lung, breast, colorectal, and bladder tumors, for example) are unfortunately poorly responsive to these agents. For both drugs, acute suppression of bone marrow activity and cumulative dose-related cardiac toxicity are dose-limiting side effects; nausea and vomiting due to acute toxicity to the gastrointestinal mucosa are almost universal following standard bolus administration; and serious ulcerating necrotic lesions can result from accidental subcutaneous drug extravasation during intravenous dosing. These limitations continue to demonstrate the need for analogues of **1** and **2** with improved therapeutic properties.



1 (adriamycin): R_1 = OH; R_2 = H
2 (daunorubicin): R_1 = R_2 = H
3 (AD 32): R_1 = $OCO(CH_2)_3CH_3$; R_2 = $COCF_3$
4: R_1 = OH, R_2 = $COCF_3$



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In the search for such analogues, these laboratories have been responsible for the conceptualization, synthesis, preclinical development, and clinical introduction of the novel analogue *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32, **3**).[6,7] In marked contrast to **1** and **2**, **3** does not bind with double-helical DNA.[8,9] Consequently, the range of biochemical and biological properties exhibited by **3** in in vitro and in vivo systems, often with effects superior to those of **1** under similar conditions,[10–21] cannot be explained

(1) The major portion of the work described in this report was done at the Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.
(2) A preliminary report on this work has appeared: Israel, M.; Seshadri, R.; Khetarpal, V. K.; Anderson, L. B.; Potti, G. Abstracts, 12th International Congress on Chemotherapy, Florence, Italy, 1981; p 73.

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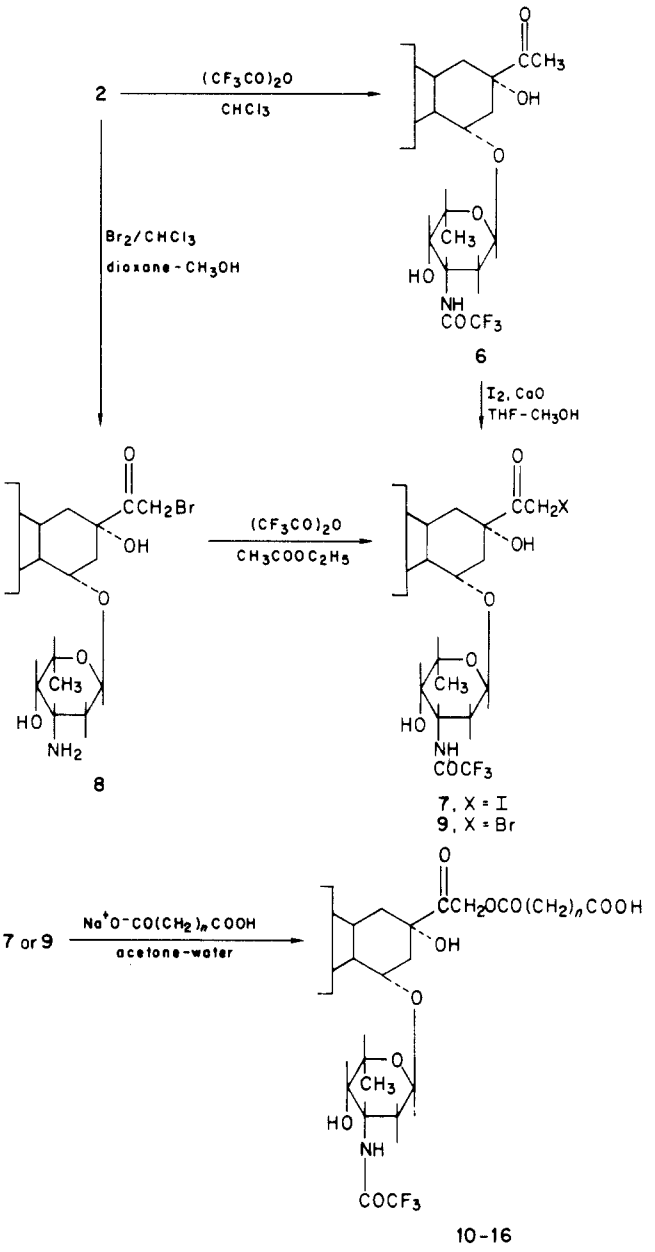
by the widely held drug–DNA binding hypothesis usually advanced as the mechanism of action for 1.[4,22,23] Compound 3 is, thus, of more-than-passing mechanistic interest.

From the practical point of view, the therapeutic superiority of 3 compared to 1 in animal model systems,[6,7,24-26] its greatly reduced toxicity,[6,24,27-29] and other potential pharmacologic advantages relating to cell penetration[30] and metabolism and disposition[31-34] led to early clinical trials, wherein activity was demonstrated for 3 against several types of human solid tumors. As for 1 and 2, bone marrow suppression is the acute dose-limiting toxicity. Nevertheless, when used at its maximally tolerated dose, 3 produced no clinical evidence of cardiotoxicity, regardless of the total accumulated dose achieved (in one instance as high as 16.5 g/m^2); gastrointestinal toxicity was greatly reduced; and drug extravasation did not result in local tissue damage.[35-37]

Scheme I



Intravenous administration of 3 is, however, complicated by its high lipophilic character and resultant water insolubility. For clinical use, the drug must be formulated at high dilution in a surfactant-containing vehicle (0.35–0.47 mg/mL of 3 in a polyethoxylated castor oil[38]–ethanol–saline mixture, 0.5%:0.5%:99%). Thus, at the usual dose of 600 mg/m^2 of 3, a patient with a body surface area of 1.0 m^2 would receive about 1.5 L of infusate. These large volumes dictate that 3 be given as a continuous infusion, generally lasting from 18–24 h, an arrangement that is inconvenient for the clinical staff, who must be available to maintain pumps and intravenous lines, and for the patient, who must remain hospitalized overnight. Furthermore, use of these polyethoxylated castor oil vehicles is associated with an unfortunate chest pain symptom complex, for which steroids (hydrocortisone hemisuccinate[39]) must be used prophylactically. While routine

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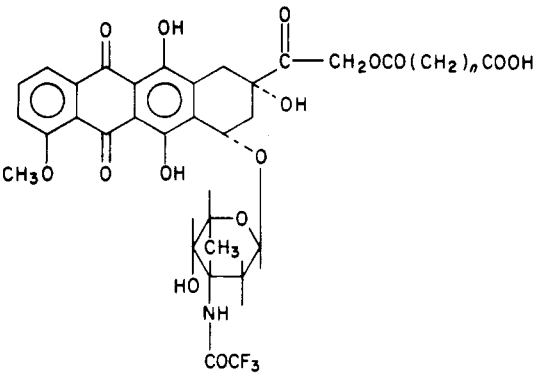
(38) Emulphor EL-620 or Cremaphor; both of these surfactants were provided as 1:1 mixtures with ethanol by the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute.
(39) Solu-Cortef (Upjohn).

steroid prophylaxis completely masks the chest pain symptomatology, the desire to move away from the use of steroids, and from the requirement of continuous infusion drug delivery, has led to the preparation and evaluation of analogues of **3** with improved aqueous solubility.

The design of the title compounds of this report is based upon pharmacological considerations relating to **3**. Compared to **1**, **3** is much more rapidly and extensively metabolized. The principal metabolic products, *N*-(trifluoroacetyl)adriamycin (**4**) and *N*-(trifluoroacetyl)adriamycinol (**5**), are rapidly excreted, primarily via the hepatobiliary pathway.[31-34,40] While **1** can be found as a minor metabolite of **3**,[41-43] the amounts of **1** derived from **3** are too small to explain the therapeutic and other biological effects exhibited by **3**. On the other hand, **3** is so rapidly metabolized in vivo, especially in rodents, that the parent drug appears unlikely to be responsible for all of the observed antitumor activity with this agent. It is possible, therefore, that **4**, or **5**, may play a significant role in the mechanism of action of **3**. In vivo, **4** is derived from **3** by the action of nonspecific plasma and intracellular esterases. Compound **5** arises from **4** by the action of ubiquitously occurring aldo–keto reductases (analogous to the conversion of **1** to its principal metabolite adriamycinol). Of the two compounds, **4** appears to be the more likely candidate for activity: **4** is more effective than **1** in prolonging the survival of mice bearing either the L1210 or P388 leukemia, whereas **5** is generally less effective than **1**. Compound **4** is inferior to **3** as an antitumor agent, in terms of its direct administration to tumor-bearing animals, and **4** reproducibly leads to late deaths of treated animals, due to delayed toxicity not seen with **3**.[44] Futhermore, **4** is even less soluble than **3**, a factor that makes it additionally unattractive for development as a drug for human use. On the basis of the consideration that **3** may be serving as a prodrug of **4**, it seemed reasonable that analogues of **3** equally capable of in vivo conversion into **4** should continue to exhibit pharmacologic properties similar to **3**; the introduction of polar groups into such analogues could provide the functionality needed for improving aqueous solubility. In the present instance, the products were designed so as to make use of an available carboxylic acid group for dissolution of the resulting compounds in dilute base.

The preparative route to the seven homologous *N*-(trifluoroacetyl)adriamycin 14-O-hemiester products described in this report is outlined in Scheme I. Daunorubicin (**2**) was converted in almost quantitative yield into *N*-(trifluoroacetyl)daunorubicin (**6**) by treatment with trifluoroacetic anhydride, using a modification of a procedure originally described in the patent literature. Compound **6** was converted into its 14-iodo derivative **7**, as previously described.[45] Alternatively, **2** was brominated selectively at the 14-position to give 14-bromodaunorubicin (**8**) as the mixed hydrochloride/hydrobromide salt. Treatment of **8** with trifluoroacetic anhydride afforded the 14-bromotrifluoroacetamide **9**.[46] Reaction of either **7** or **9** with the monosodium salts of dibasic acids (malonic, succinic, glutaric, adipic, pimelic, azelaic, sebacic) in boiling acetone gave the desired 14-O-hemiesters **10–16**. During the course of the reaction, small amounts of **4** and aglycones were also formed. The hemiester products were purified by column chromatography on silicic acid, with chloroform containing

Table I. In Vitro and in Vivo Bioassay Data for *N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiester Products



10-16

no.	*n*	cell growth inhibn: ID_{50},[a] μM	in vivo antitumor act.[b] dose, mg/kg	% ILS[c]	60-day survivors
1		0.05	1.0	+81	0/7
			2.0	+90	0/7
			3.0	+181	0/7
			4.0	+63	0/7
			5.0	-19	0/7
3		0.24	20.0	+281	3/7
			30.0	>+445	4/7
			40.0	>+445	6/7
			50.0	>+445	5/7
			60.0	>+445	5/7
10	1	0.33	40.0	+109	0/7
			50.0	+109	0/7
			60.0	+136	1/7
			70.0	+154	1/7
11	2	0.30	40.0	+127	0/7
			50.0	+118	0/7
			60.0	+127	0/7
			70.0	+190	1/7
12	3	0.28	30.0	+81	0/7
			40.0	+109	1/7
			50.0	+172	2/7
			60.0	>+445	4/7
			70.0	>+445	4/7
13	4	0.31	40.0	>+445	6/7
			50.0	>+445	5/7
			60.0	>+445	6/7
			70.0	>+445	7/7
14	5	0.36	40.0	+181	3/7
			50.0	+136	1/7
			60.0	>+445	7/7
			70.0	>+445	6/7
15	7	0.44	30.0	+445	4/7
			40.0	+172	3/7
			50.0	>+445	5/7
			60.0	>+445	5/7
			70.0	>+445	7/7
16	8	0.50	40.0	+109	0/7
			50.0	>+445	4/7
			60.0	>+445	5/7
			70.0	>+445	6/7

[a] Versus CCRF-CEM cells in culture; 48-h incubation. [b] Versus murine P388 leukemia; B6D2F1/J male mice inoculated with 10^6 tumor cells ip on day 0; treatment once daily on days 1–4 ip. [c] Percent increase in life span relative to untreated controls; median of zero-dose controls, 11 days (range 10–14).

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increasing amounts of methanol as the liquid phase. Yields were usually somewhat higher from **7** than from **9**. Products were fully characterized by spectral features and microchemical analyses.

In vitro and in vivo bioassay data for the seven hemiester products prepared here, together with relevant data for **1** and **3**, are given in Table I. Target compounds were first examined in vitro for their ability to inhibit the growth of CCRF-CEM cells, a lymphoblastic leukemic cell line of human origin. Assay conditions have been previously described.[47] The ID_{50} value represents the concentration of drug necessary to inhibit the growth of cultures by 50% relative to untreated controls. As noted in the table, the ID_{50} values for the hemiester products are of the same order as those for **3** and **4** (ID_{50} = 0.23 uM), suggesting that the new compounds may be exerting their cytotoxic action via a mechanism in common with that of **3** and **4**. The decreasing activity of the longer chain products **15** and **16** may be a reflection of increasing drug–serum protein binding, an effect analogous with that seen earlier by us with a more extensive homologous series of *N*-(trifluoroacetyl)adriamycin-14-alkanoates.[7] We believe that the high activity of **1** in this assay is less a reflection of its antitumor activity than it is a measure of the drug's in vivo toxicity.

In vivo antitumor activity of the target compounds was evaluated in B6D2F1 male mice bearing the murine P388 leukemia (10^6 tumor cells implanted intraperitoneally on day 0), following the standard protocols of the National Cancer Institute.[48] Treatment was begun on the first day after tumor implantation and was continued once daily for four consecutive days. In initial therapy trials, experiments were designed to compare the activities of the hemiester compounds with that of **3** under similar conditions. As a result, all test compounds, as well as **3** as a positive control, were administered intraperitoneally to tumor-bearing animals as solutions in 10% polyethoxylated castor oil–10% ethanol–80% saline; compound **1**, in 0.9% saline, was included as an additional positive control. In previous studies here and elsewhere, **3** has shown dramatic therapeutic superiority to **1** under these assay conditions;[6,7,24,25] no difference in activity is seen with **1** formulated in surfactant-containing vehicle as compared to saline.

The results shown in Table I are from a single internally consistent experiment. For the two positive controls, **3** was, as usual, demonstrably superior to **1** in therapeutic activity; the results with these known agents, consistent with historical data, thus provide legitimacy for the findings with the test hemiester products. Significant prolongation of survival of tumor-bearing mice was seen with all of the hemiester target compounds. The hemimalonate product **10** was essentially equivalent in activity with **1**, each at its optimal dose. All of the other test compounds were clearly superior to **1**, with several showing equivalence in curative properties with **3**. The hemiadipate derivative **13** appeared from this experiment to be the best compound of the series, with the broadest dose–response range for curative activity. The high in vivo antitumor activity of various of these hemiester derivatives, including **13**, vs. the murine P388 tumor has been confirmed in repeat assays.

With respect to solubility, it should be noted that the solubility of **3** in water, or pH 7.2 Tris or phosphate buffer, is less than 1 μg/mL, an amount too little to even show color. In Tris pH 7.2 buffer containing 2.5% ethanol, the solubility of **3** increases to 25 μg/mL. For animal studies, **3** must be formulated in a surfactant-containing vehicle [10% aqueous Tween (polysorbate) 80 or 10% polyethoxylated castor oil–10% ethanol–80% saline]. In clinical practice, only the polyethoxylated castor oil formulations, with the limitations previously noted, have proven practicable for **3**.

Table II. Solubility and Stability of *N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiester Products

no.	solvent syst[a]	sol, mg/mL	24-h stability[b] 4 °C	24-h stability[b] 27 °C
10	A	neglig[c]		
	B	neglig[c]		
	C	neglig[c]		
	D	>69	0 (stable)	trace
	E	*d*	1	3
	F	*d*	2.5	6
11	A	neglig		
	B	neglig		
	C	neglig		
	D	>68	1	2
	E	*d*		
12	A	insol		
	B	insol		
	C	insol		
	D	65	0 (stable)	1
	E	70	<0.3	3
13	A	insol		
	B	insol		
	C	insol		
	D	60	0 (stable)	~0.5[e]
	E	65	0.3	3
	F	70	0.5	
14	A	insol		
	B	insol		
	C	insol		
	D	15	0 (stable)	~0.5
15	D	10		
16	D	10		

[a] A = demineralized water; B = 0.9% sodium chloride; C, D, E = commercial potassium phosphate monobasic–sodium hydroxide buffer, 0.05 M, pH 6.00, 7.40, and 8.00 respectively; F = commercial boric acid–potassium chloride–sodium hydroxide buffer, 0.1 M, pH 9.00. [b] Expressed as percent hydrolytic conversion to *N*-(trifluoroacetyl)adriamycin over 24 h in the dark, as determined by TLC and/or HPLC; no other decomposition products observed. [c] Solubility less than 50 μg/mL, barely enough to give solution faint pink-orange tinge. [d] Readily soluble; insufficient compound available for precise quantitation. [e] ~3% over 72 h.

The improvement in solubility over **3** offered by these hemiester analogues is shown in Table II, which gives data on the solubility of the compounds in aqueous medium, in some instances across a range of pH. Stability of solutions was monitored by thin-layer and high-performance liquid chromatographic assays. Because of the available carboxylic acid function, solubility was seen to increase, as would be expected, with increasing pH. However, also as expected, stability, relative to base-catalyzed hydrolysis of the ester linkage, decreased with increasing pH. The hydrolytic reaction was more pronounced at room temperature than at 4 °C. No other degradation products were noted with any of the compounds in these studies. For **13** the increase in aqueous solubility approaches a factor of 10^5 relative to **3**.

Of the series of seven hemiester derivatives of **4** reported here, the hemiadipate **13**, which is markedly active against the murine P388 leukemia in vivo, also exhibits the best characteristics of solubility and stability at pH 7.4. This compound has emerged as our analogue of choice for further development. Additional studies with **13** in the murine P388 leukemia system have shown that the superior antitumor effects of **13** and **3** vs. **1** are not due to any contribution by the surfactant formulation: when **13** was

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formulated in surfactant vehicle for comparison with 3, and compared in the same experiment with 13 prepared in aqueous pH 7.4 phosphate buffer and with 1 formulated in saline, survival data obtained for 1, 3, and 13 were similar to those shown for these agents in Table I, regardless of the vehicle used to formulate 13.

On the basis of the advantageous pharmacological properties of 13, as described in this report, this compound, and its next lower homologue 12, also significantly biologically active, were selected for patent coverage.[49] A preliminary report showing the therapeutic superiority of 13 over 1 in several murine solid tumor models has since appeared.[50] Attempts are currently under way to bring 13 to early clinical trial as a second-generation analogue of 3 with improved water solubility. Assuming an equivalent dose-response and toxicity with 3 in humans, as appears to be the case for the two drugs in the mouse, administration of a clinical course of 13 might be possible as a 10–20 mL bolus of an entirely aqueous formulation, instead of the surfactant-containing vehicle, 24-h infusion, and steroid prophylaxis needed with 3.

Experimental Section

Monosodium salts of the dibasic acids used in this work were prepared by carefully titrating the acid with 1 equiv of sodium hydroxide and lyophilizing the resulting mixture. Infrared spectra were recorded as KBr disks on a Perkin-Elmer Model 137B spectrophotometer. Absorption spectra were determined in methanol on a Varian Associates Cary Model 15 UV-vis spectrophotometer. Optical rotational data were obtained spectrometrically on a Perkin-Elmer spectropolarimeter. Proton NMR spectra were recorded on a Varian Associates Model T60-A spectrometer in chloroform, with tetramethylsilane as internal standard; spectral data were consistent with expected structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN; found values are within ±0.4% of theory.

14-Bromodaunorubicin (8). This procedure is a modification of that described in the patent literature.[45] Daunorubicin hydrochloride (900 mg, 1.59 mmol) was dissolved in dioxane (67 mL, freshly distilled from sodium) and methanol (31 mL, feshly distilled from magnesium turnings). To this solution was added dropwise a solution of bromine in chloroform (7.8 mL, 4.6 g of bromine diluted to 50 mL with chloroform) over a 10-min period. The reaction mixture was stirred at room temperature for 2 h. The solution was poured into anhydrous ether (500 mL), with stirring, and the precipitated material was filtered immediately, washed with anhydrous ether (100 mL), and dried. The red solid was taken into a mixture of 1 N aqueous HBr (36 mL) and acetone (36 mL), and the solution was kept in the refrigerator for 12 h. After dilution with water (40 mL), the small amount of aglycone present was separated by extraction twice with chloroform (100 mL). The aqueous solution was treated with saturated NaCl solution, and the bromo derivative 8 was extracted from the aqueous phase with 1-butanol (20 mL × 4). The extract was evaporated to a small volume (5 mL) under reduced pressure. The product was precipitated by the addition of petroleum ether (bp 60–90 °C), separated by filtration, and dried (892 mg, 88% yield).

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemimalonate (10).** A mixture of *N*-(trifluoroacetyl)-14-iododaunorubicin (7; 150 mg, 0.2 mmol) and malonic acid monosodium salt (1 g) was taken in water (2 mL) and then diluted with acetone (300 mL). The reaction mixture was stirred and heated at reflux for 14 h. After cooling, the reaction mixture was filtered, and the filter cake was washed with hot acetone until the washings were no longer colored. The combined filtrate and washings were evaporated to dryness under reduced pressure, and the residue was redissolved in chloroform (200 mL). The chloroform solution was washed three times with 150-mL portions of water, dried over anhydrous Na_2SO_4, filtered, and evaporated to about 2–3-mL volume. This concentrate was chromatographed on a column of silicic acid (Biosil A; Biorad) using chloroform, followed by 0.5% methanol in chloroform as eluant. A concentrated chloroform solution of the eluted product was triturated with petroleum ether to give 10 (123 mg, 84%) as a red solid; mp 145–148 °C dec; $[\alpha]_D$ +198° (*c* 0.02, CH_3OH); IR 3475 (br, OH, HOCO–), 1742, 1730, 1718, 1670 (C=O and quinone) cm^{-1}; UV-vis λ_{max} (ϵ) 232 nm (32 500), 242 (23 400), 275 (12 000), 477 (9390)496 (9150), 530 (5300). Anal. ($C_{32}H_{30}F_3NO_{15}$) C, H, F, N.

The same product was obtained in 59% yield when bromo compound 9 was used as starting material.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemisuccinate (11).** A suspension of 7 (300 mg, 0.4 mmol) and succinic acid monosodium salt (2 g) in acetone (350 mL) containing 3 mL of water was heated at reflux for 12 h. The same workup procedure as in the previous example was used to give 11 (192 mg, 65%): mp 150–152 °C dec; $[\alpha]_D$ +212° (*c* 0.046, CH_3OH); UV-vis λ_{max} (ϵ) 232 nm (32 400) 239 (22 800), 275 (10 300), 476 (9830), 496 (9590), 531 (5270). Anal. ($C_{33}H_{32}F_3NO_{15}$) C, H, F, N.

In another experiment, 7 was replaced by the corresponding bromo derivative 9; the reaction gave 62% yield of product identical in all respects with the material described above.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiglutarate (12).** A mixture of 7 (200 mg, 0.26 mmol) and glutaric acid monosodium salt (1.2 g) was taken up in 3 mL of water and then was diluted with acetone (350 mL). The reaction mixture was heated at reflux for 10 h. Similar workup as for 10 resulted in 12 (152 mg, 76%): mp 141–144 °C dec; $[\alpha]_D$ +288° (*c* 0.046, CH_3OH); UV-vis λ_{max} (ϵ) 233 nm (29 700), 240 (20 800), 277 (9900), 478 (8540), 495 (8350), 531 (4270); IR 3450 (br, OH, HOCO–), 1715, 1705, 1620, 1580 (C=O and quinone) cm^{-1}. Anal. ($C_{34}H_{34}F_3NO_{15}$) C, H, F, N.

The hemiglutarate 12 was obtained in a yield of 60% when the starting iodo derivative was replaced by 14-bromo compound 9.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiadipate (13).** A mixture of 7 (150 mg, 0.2 mmol) and adipic acid monosodium salt (1 g) was taken up in 300 mL of acetone containing 2 mL of water. The reaction mixture was heated at reflux for 12 h. Similar workup as before gave 13 (133 mg, 85%) as a red solid: mp 135–140 °C dec; $[\alpha]_D$ +201° (*c* 0.016, CH_3OH); IR 3460 (br, OH, HOCO–), 1720, 1710, 1680, 1650 (C=O and quinone) cm^{-1}; UV-vis λ_{max} (ϵ) 233 nm (33 200), 241 (23 800), 276 (9020), 478 (9780), 497 (9590), 532 (4990). Anal. ($C_{35}H_{36}F_3NO_{15}$) C, H, F, N.

Alternatively, when 9 was substituted for 7 as starting material, the hemiadipate was obtained in 64% yield.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemipimelate (14).** A mixture of 7 (250 mg, 0.34 mmol) and pimelic acid monosodium salt (1.3 g) was taken up into 300 mL of acetone containing 2 mL of water. The reaction mixture was stirred and heated at reflux for 14 h. Isolation and purification of the product, as for 10, afforded 14 (174 mg, 67%): mp 140–145 °C dec; $[\alpha]_D$ + 267° (*c* 0.022, CH_3OH); IR 3445 (br, OH, NH), 1738, 1630, 1600 (carbonyls) cm^{-1}; UV-vis λ_{max} (ϵ) 232 nm (34 650), 240 (25 200), 274 (12 800), 476 (9580), 496 (9265), 530 (5400). Anal. ($C_{36}H_{38}F_3NO_{15}$) C, H, F, N.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemiazelate (15).** Reaction of 300 mg (0.4 mmol) of 7 with 1.5 g of azelaic acid monosodium salt in 350 mL of refluxing acetone containing 3 mL of water, followed by purification as in previous examples, afforded 15 (210 mg, 65%): mp 120–125 °C dec; $[\alpha]_D$ +258° (*c* 0.02, CH_3OH); IR 3439 (br, OH and NH), 1737, 1638, 1605 (carbonyls) cm^{-1}; UV-vis λ_{max} (ϵ) 233 nm (34 800), 241 (25 800), 275 (11 850), 477 (9950), 497 (9780), 531 (5200). Anal. ($C_{38}H_{42}F_3NO_{15}$) C, H, F, N.

***N*-(Trifluoroacetyl)adriamycin 14-*O*-Hemisebacate (16).** Reaction of 350 mg (0.48 mmol) of 7 with sebacic acid monosodium salt (1.8 g) in 400 mL of acetone containing 3 mL of water at reflux for 14 h, followed by isolation and purification as before, afforded 16 (242 mg, 63%): mp 108–112 °C dec; $[\alpha]_D$ +217° (*c* 0.022, CH_3OH); UV-vis λ_{max} (ϵ) 232 nm (32 300), 240 (21 200), 277 (11 200), 478 (9830), 495 (9640), 531 (5140). Anal. ($C_{39}H_{44}F_3NO_{15}$) C, H, F, N.

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Synthesis and Antimetastatic Properties of Stereoisomeric Tricyclic Bis(dioxopiperazines) in the Lewis Lung Carcinoma Model[1]

Donald T. Witiak,*[†] Raghunathan V. Nair,[†] and Franz A. Schmid[‡]

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and Laboratory of Experimental Antitumor Testing, Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye, New York 10580. *Received November 14, 1985*

Synthesis of *trans*- and *cis*-tetrahydrodipyrazino[1,2-*a*:1′,2′-*d*]pyrazine-1,3,7,9(2*H*,4*H*,8*H*,10*H*)-tetrone analogues **10** and **11** belonging to the bis(dioxopiperazine) class of antitumor agents and their bis(morpholinomethyl) derivatives **12** and **13** are described with use of 2,5-dimethylpyrazine as the starting material. Synthetic studies utilizing 3,6-disubstituted 2,5-dioxopiperazine precursors are included. Evaluation of **10–13** in the Lewis Lung carcinoma model indicated the bis(morpholinomethyl) analogue *cis*-**13** to be antimetastatic, whereas the trans isomer **12** was toxic at a similar dose effecting a decrease in the life span of treated mice. The parent bis(dioxopiperazines) **10** and **11** were ineffective as antitumor or antimetastatic drugs.

Chemical, biochemical, and pharmacological properties of antitumor bis(dioxopiperazines) recently have been reviewed.[2] Cyclic analogues **4–9** of **1** and **2** exhibited stereoselective effects in various tumor models.[3-6] In the solid state a cis "face to face" conformation of the dioxopiperazine rings were observed in antimetastatic racemic **2** and *cis*-**5**.[7,8] However, such a conformation seems not to be essential for activity since tricyclic bis(dioxopiperazine) *trans*-**6** exhibited antimetastatic properties in the B16-F10 melanoma model.[5,9]

Recent reports from China[10] have indicated the bis(morpholinomethyl) derivative of **1** (namely, **3**) to be effective in various human malignancies. Although such compounds are predictably unstable and undergo hydrolysis to the parent dioxopiperazines, morpholinomethyl–N groups may impart antineoplastic properties to a molecule owing to possible alkylating activities not unlike those proposed[11] for certain hydroxymethyl–N metabolites of therapeutically useful drugs. Comparative analysis of morpholinomethyl derivatives **8** and **9** with the respective parent imides **6** and **7** in a postoperative Lewis Lung (LL) carcinoma model revealed morpholinomethyl cis-syn-trans isomer **9** to be more effective as an inhibitor of metastasis than the other three analogues (**6–8**).[12] Additionally, the order of decreasing activity was **9** > **8** > **7** > **6** when assessed in terms of survival or antimetastatic data. The increased activity observed for the morpholinomethyl derivatives over their respective parents was attributed[12] either to increased solubility and drug delivery (prodrug) or to an intrinsic antitumor activity of the morpholinomethyl–N functionality possibly reflecting macromolecular alkylation. In this report we describe the synthesis of analogues **10–13** and biological evaluation of these compounds in the LL carcinoma model using the postoperative protocol. Such studies are of interest since previous investigations of conformationally constrained analogue pairs (**4**, **5** and **6**, **7**) suggested that a "cisoid" relationship of imide functionalities was necessary for antimetastatic activity.[4-6] Clearly, interatomic distances between groups in **10** and **11** are markedly different than those found in **6** and **7**. Consequently, selective antitumor activity observed for morpholinomethyl analogues would provide support for intrinsic antitumor activity of the morpholinomethyl–N functionality.

Chemistry. Our synthetic strategy utilized the analysis shown in Scheme I. Two pathways were explored for the synthesis of piperazines **20–22**. One involved selective reduction of the amide functionalities in 3,6-disubstituted 2,5-dioxopiperazine precursors. Dioxopiperazine diester **23** of undefined stereochemistry was reported[13] to have been formed on cooling a solution of ethyl aminomalonate

[†] The Ohio State University.
[‡] Sloan-Kettering Institute for Cancer Research.

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