derivatives to substrate prior to the addition of membranes. The specific binding was then measured, IC_{50} concentrations were determined graphically, and K_D values were calculated as described.[21]

Acknowledgment. The authors appreciate the experimental assistance of Dr. Jerzy Langer, Barbara Hughes, and Gersham Wilkerson and thank Deborah Lamartin for improvements and typing of the manuscript. The advice and support of Dr. Ronald R. Tuttle is also gratefully acknowledged.

Registry No. **1a**, 83650-73-1; **2a**, 83650-74-2; **3a**, 83650-75-3; **3b**, 83650-77-5; **3c**, 83650-79-7; **3d**, 83650-81-1; **4a**, 83650-76-4; **4b**, 83650-78-6; **4c**, 83650-80-0; **4d**, 83681-19-0; **5a**, 4638-04-4; **5d**, 2461-42-9; 1-chloro-2,3-epoxypropane, 106-89-8; *o*-allylphenol, 1745-81-9; α-naphthol, 90-15-3; jeffamine, 9046-10-0.

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Adriamycin Analogues. Preparation and Biological Evaluation of Some Novel 14-Thiaadriamycins[1]

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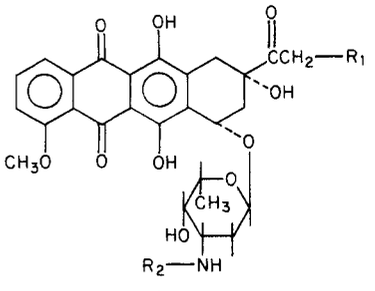
Condensation of 14-bromodaunorubicin with thiols in methanol, in the presence of potassium carbonate, resulted in the formation of 14-thia analogues of the antitumor antibiotic adriamycin. However, similar condensation of *N*-(trifluoroacetyl)-14-iododaunorubicin with thiols invariably led to a redox reaction, with the formation of *N*-(trifluoroacetyl)daunorubicin and disulfides. Accordingly, *N*-(trifluoroacetyl)-14-bromodaunorubicin was used for reaction with thiols to yield thia analogues of the clinically active but non-DNA-binding adriamycin analogue *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32). Reaction of 14-bromodaunorubicin with α,ω-alkanedithiols gave bis(thiaadriamycin) analogues as potential difunctional intercalating agents. The aforementioned products, plus two related phenylselena derivatives, were examined for in vitro growth inhibition, in vivo antitumor activity, and, where appropriate, DNA binding. A number of agents, most notably 14-(carbethoxymethyl)-14-thiaadriamycin and *N*-(trifluoroacetyl)-14-phenyl-14-selenaadriamycin, were active against murine L1210 leukemia in vivo. Several of the amino glycoside unsubstituted 14-thiaadriamycin analogues exhibited DNA-binding properties equivalent to those of adriamycin.

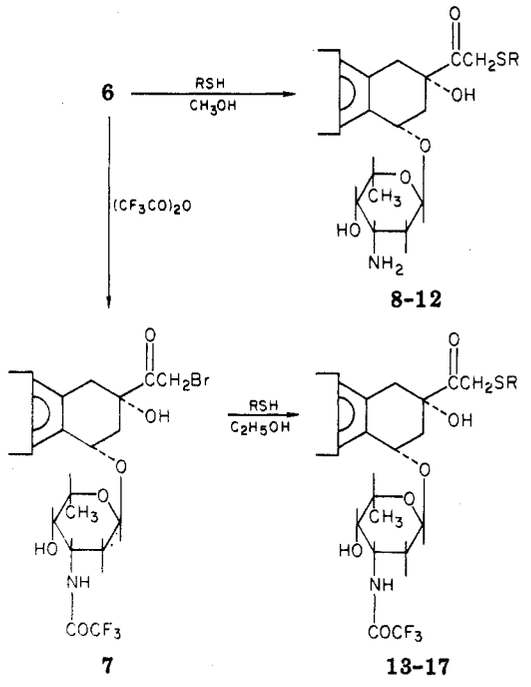
For some time these laboratories have been involved in the search for analogues of the clinically important antitumor antibiotics daunorubicin (**1**) and adriamycin (**2**) and



1, R_1 = H; R_2 = H (daunorubicin)
2, R_1 = OH; R_2 = H (adriamycin)
3, R_1 = $OCO(CH_2)_3CH_3$; R_2 = $COCF_3$
4, R_1 = I; R_2 = $COCF_3$
5, R_1 = H; R_2 = $COCF_3$
6, R_1 = Br; R_2 = H
7, R_1 = Br; R_2 = $COCF_3$

in the determination of the structure–activity correlates among this family of compounds. In connection with this program, we have now prepared a number of hitherto unknown 14-thia- and 14-selenaadriamycin derivatives and have evaluated these products for in vitro cell growth inhibitory activity, for in vivo antileukemic activity in a murine tumor model, and, where appropriate, for their ability to interact with DNA. Like the parent agents (**1** and **2**), some of the target compounds reported here possess an unsubstituted glycosidic amino function, a molecular feature claimed to be essential for DNA binding and resultant antitumor activity.[2-4] These products in-

Scheme I



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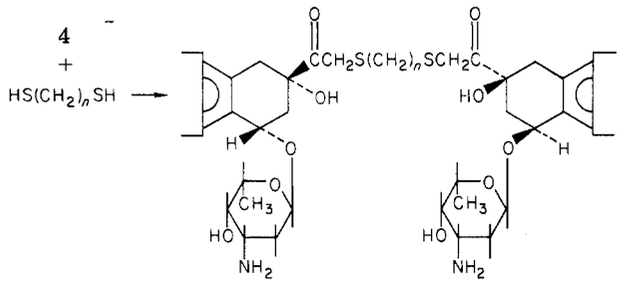
Scheme II



20, $n = 2$
21, $n = 3$
22, $n = 6$
23, $n = 9$

clude several bis(thiaadriamycin)s, which have been made as potential difunctional DNA intercalating agents. *N*-(Trifluoroacetyl)-14-thiaadriamycins have also been prepared and studied, based upon the therapeutic superiority in experimental systems and lesser toxicity, including lesser cardiac toxicity, of *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32; **3**). Compound **3** is a non-DNA-binding adriamycin analogue first prepared and developed in these laboratories and currently showing clinical activity in phase I/II trials.[5-16]. While the mechanism of action of **3** remains speculative, it is clear from a range of pharmacology and cell biology studies[7,11,12,15,17-21] that its biological properties are not due simply to a prodrug effect. It is hoped that the study of structure–activity relationships among *N*-(trifluoroacetyl)anthracyclines, relative to their unsubstituted amino glycoside counterparts, may help shed light on the mechanism of action of **3**.

Chemistry. The 14-thiaadriamycin analogues (**8–17**) and the bis(thiaadriamycin)s (**20–23**) were prepared according to the reaction sequences shown in Schemes I and II.

Initial attempts to prepare *N*-(trifluoroacetyl)-14-thiaadriamycin analogues involved reaction of *N*-(trifluoroacetyl)-14-iododaunorubicin (**4**)[22] with a variety of alkyl and aryl thiols. These reactions invariably led to the instantaneous formation of *N*-(trifluoroacetyl)daunorubicin (**5**) identified by comparison of properties (IR, NMR, TLC, and HPLC) with an authentic sample. Further studies with various model compounds have established the generality of the reduction of iodomethyl ketones to methyl ketones by thiols (and selenols) in high, often quantitative, yield under mild conditions; this work is described elsewhere.[23]

In contrast to **4**, the aglycon 14-bromodaunomycinone,[22] used as a model compound, was found to react smoothly with thiols (ethyl mercaptoacetate, dodecanethiol, and benzenethiol) to give the corresponding 14-thiaadriamycinones. Based on these observations, 14-bromodaunorubicin (**6**)[22] was tried and used successfully for the preparation of the desired 14-thia analogues **8–12**. Reactions were run in methanol or ethanol at room temperature for 30 min and proceeded in high yield.

Complicatons observed in the attempted trifluoroacetylation of some of the thiaadriamycin products, **8–12**, led us to convert **6** into its hitherto unknown trifluoroacetamide **7**, which was then used to prepare **13–17**. Compound **7** was prepared from **6** in the same way that **5** is obtained from **1**.[22] Although purified **7** (chromatography on BioSil A) was homogeneous on TLC and HPLC, a satisfactory elemental analysis could not be obtained. However, on treatment with thiols, it yielded the desired sulfides, unambiguously characterized by spectral data and elemental analyses. This led us to consider that **7** was probably a mixed halide (Br and Cl), a conclusion which was substantiated by further elemental analysis, which showed the presence of both halogens.

In like manner, two selenium analogues, 14-phenyl-14-selenaadriamycin (**18**) and *N*-(trifluoroacetyl)-14-phenyl-14-selenaadriamycin (**19**), were also prepared.

The bis(thiaadriamycin) analogues **20–23** were prepared from **6** by reaction with α,ω-alkanedithiols, as described. In all instances, products were purified by open-column chromatography on BioSil A, checked for homogeneity by TLC and HPLC, and characterized by IR and NMR spectral data and elemental analyses.

Biological Evaluation. The structures of the simple alkythio products prepared during this investigation and their in vitro and in vivo biological properties are shown in Table I, together with data for **2** and **3** for purposes of comparison.

The ability of compounds **8–12** to bind to calf thymus DNA was determined spectrophotometrically at 480 nm by the spectral shift method.[24,25] The ratio of the optical densities of drug in the presence of DNA (A) to drug alone (A_0) is a measure of the extent of binding, with a value of unity indicating no DNA interaction. By this method, adriamycin, for comparison, shows an A/A_0 value of 0.60, and **3** shows a value of 0.96. As shown in Table I, the extent of binding of several of the 14-thiaadriamycin

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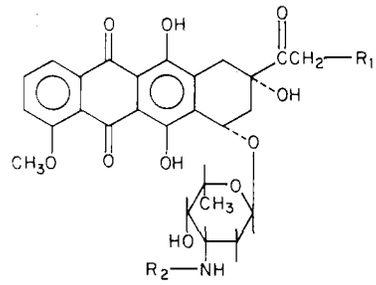
Table I. Biological Evaluation of 14-Thia- and 14-Selenaadriamycin Analogues



no.	R_1	R_2	extent of DNA binding: A/A_0 (λ 480 nm)[a]	cell growth inhibn: ID_{50},[b] μM	in vivo antitumor act.[c] opt dose,[d] mg/kg	in vivo antitumor act.[c] % ILS[e]
2	OH	H	0.60[f]	0.066[g]	4.0	+50[g]
3	$OCO(CH_2)_3CH_3$	$COCF_3$	0.96[f]	0.24[g]	50.0	>+455[g]
8	$S(CH_2)_3CH_3$	H	0.60	0.25	10.13	+33
9	$S(CH_2)_4CH_3$	H	0.60	0.95	10.13	+33
10	$S(CH_2)_{11}CH_3$	H	0.84	1.44	10.13	+11
11	SC_6H_5	H	0 68	0.87	10.13	+22
12	$SCH_2COOC_2H_5$	H	0.56	0.23	30.0	+50
18	SeC_6H_5	H		0.40		
13	$S(CH_2)_3CH_3$	$COCF_3$		5.62	67.5	0
14	$S(CH_2)_4CH_3$	$COCF_3$		>13.80	67.5	+10
15	$S(CH_2)_{11}CH_3$	$COCF_3$		>15.00	67.5	0
16	SC_6H_5	$COCF_3$		>13.70	101.25	+10
17	$SCH_2COOC_2H_5$	$COCF_3$		5.33	67.5	0
19[h]	SeC_6H_5	$COCF_3$		2.80	60.0	+66

[a] Calf thymus DNA; pH 7.0 Tris buffer; drug/DNA-P molar ratio = 0.1. [b] vs. CCRF-CEM (human lymphoblastic leukemic) cells in culture. [c] vs. murine L1210 leukemia; $B6D2F_1J$ male mice inoculated with 10^5 tumor cells ip on day 0; treatment once daily on days 1-4 ip. [d] Highest nontoxic dose. [e] Percent increase in life span relative to untreated controls. [f] Reference 9. [g] Reference 5. [h] Synthesis and chemical properties described in ref 23.

analogues described here is as great as, or greater than, that of the parent antibiotic. The trifluoroacetamides were not assayed for DNA complexation, based upon well-established structure–activity considerations that require an unsubstituted glycosidic amino function for binding.[4,9]

Despite the significant extent of DNA binding for some of the above compounds, none of the agents was as inhibitory of the growth of cells in culture as is adriamycin. Compounds were tested for cell growth inhibitory activity against CCRF-CEM (human leukemic lymphoblast-derived) cells in culture according to previously described assay procedures.[26] The results shown in Table I indicate the dose of agent required to bring about a 50% reduction in cell growth, as compared to untreated control cultures (ID_{50} value). Several of the amino glycoside unsubstituted thia analogues were active against cultured human leukemia cells at ID_{50} values in the same range of concentration as our clinically active adriamycin analogue **3** (0.24 μM).[5] Adriamycin is, however, more growth inhibitory in this system (ID_{50} = 0.066 μM).[5] The phenylselena derivative **18** also showed significant activity against CEM cells. All of the corresponding trifluoroacetamides were inactive (ID_{50} > 1.0 μM).

In vivo antileukemic activity was evaluated in the murine L1210 system (10^5 tumor cell inoculum ip, treatment ip on days 1–4), according to the standard protocols of the National Cancer Institute.[27] Several of the target compounds showed moderate to significant antitumor activity, although none was more effective than adriamycin, which in this test system at 3.0–4.0 (mg/kg)/day for 4 days usually shows a +50 to +100% increase in life span. The activity of the selena derivative **19** is noteworthy, as none of the corresponding thiatrifluoroacetamides showed any activity. Compound **12** was also evaluated against the murine P388 leukemia (10^6 tumor inoculum ip, treatment once daily on days 1–4 ip), with a finding of a +90% increase in life span (ILS) at the optimal dose of 30 mg/kg, compared with a +136% ILS for adriamycin at a 2.0 mg/kg optimal dose.

Table II. In Vitro Biological Evaluation of Bis(thiaadriamycin)s[a]

no.	methylene bridge	ID_{50}, μM, vs. CCRF-CEM cells in culture	extent of DNA binding: A/A_0 (λ 480 nm)[b]
20	$(CH_2)_2$	1.06	0.74
21	$(CH_2)_3$	0.83	0.76
22	$(CH_2)_6$	1.03	0.85
23	$(CH_2)_9$	1.23	0.97

[a] All compounds inactive vs. L1210 leukemia in vivo. [b] Calf thymus DNA; pH 7.0 Tris buffer; drug/DNA-P molar ratio = 0.1.

The bis(thiaadriamycin) analogues, **20–23**, prepared as potential difunctional DNA-binding agents, exhibited little antitumor activity in vivo. In vitro, they were inhibitory of growth of CEM cultures at about 1 μM concentration and showed a pattern of decreasing DNA interaction with increasing length of the methylene bridge (Table II). Based on the data, it appears unlikely that these agents can serve as bis-intercalating agents.

For the limited series of compounds **8–12**, a structure–activity relationship is discernible in that increasing in vitro growth-inhibitory activity and in vivo antileukemic activity correlate with increasing DNA complexation. Overall, however, the poor activity of the reported compounds is disappointing. In view of recent considerations on the

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anthracycline mechanism of action involving superoxide and free-radical cascade reactions,[28] the low activity of 8–12, relative to 1 and 2, and the lack of activity of 13–17, relative to 3, may be the result of the sulfur atom in these products acting as a free-radical scavenger. From the structure–activity point of view, it may therefore be worthwhile to further investigate these agents, for example, for their potential effects on cultured heart cells[29] and with respect to their behavior in an anthracycline-mediated, oxygen-dependent biochemical system capable of assessing DNA damage.[30]

Experimental Section

IR spectra, recorded as KCl pellets on a Perkin-Elmer Model 137B Infracord, show characteristic bands for the anthracycline nucleus, including 3508 (OH), 1620, 1587 (quinone C=O), and 1724 cm^{-1}, wth the last-indicated signal being very much more intense in the case of the *N*-trifluoroacetyl derivatives. Proton NMR spectra were recorded in $CDCl_3$ on a Varian T60A spectrometer with Me_4Si as internal standard. All compounds showed the expected signals for the anthracycline nucleus: δ 1.29 (d, J = 6.5 Hz, 5′-CH_3), 4.1 (s, OCH_3), 5.23 (br s, 7-H), 5.52 (br s, 1′-H), 7.43–8.18 (m, aromatic H), 13.2 (s, phenolic OH), 14.0 (s, phenolic OH), and, in the case of *N*-trifluoroacetyl compounds, 6.70 (d, J = 10 Hz, $NHCOCF_3$). Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN, and are within ±0.4%, unless otherwise noted. TLC on silica gel G (Analtech), with either $CHCl_3$–MeOH–H_2O, 120:20:1 (A), or $CHCl_3$–MeOH–H_2O, 80:30:3 (B), as eluant, was chosen for identification and evaluation of homogeneity. Column chromatography was done either on BioSil A (100–200 mesh) (Bio-Rad Laboratories) or on deactivated BioSil (shaken with water and dried at 120 °C). For HPLC, 2 ft × $^1/_8$ in. OD columns packed with phenyl/corasil (Waters Associates) were used.

14-*n*-Butyl-14-thiaadriamycin (8). 14-Bromodaunorubicin (6;[22] 200 mg, 0.311 mmol) and anhydrous K_2CO_3 (50 mg, 0.368 mmol) were suspended in MeOH (20 mL). To this was added 1-butanethiol (40 μL, 0.374 mmol). After 30 min, no unreacted 6 was detectable (TLC, B). The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with pH 10 buffer (2 × 25 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and filtered, and the filtrate was evaporated to dryness; the residue was triturated free from $CHCl_3$ with pentane. The resulting solid was then chromatographed on deactivated BioSil (10 g). $CHCl_3$–MeOH (2%) eluted pure 8 (180 mg). The hydrochloride of 8 was prepared (3.1 mL of 0.1 N HCl added to $CHCl_3$ solution, followed by 2-propanol to remove water on evaporation) and then triturated with hot ethyl acetate, allowed to cool, and filtered; the residue was washed with pentane and dried to yield 8·HCl (175 mg; 87%): homogeneous on TLC (B; R_f 0.72) and HPLC (t_R = 2.01 min; CH_3CN, 55%/pH 4 ammonium formate buffer, 45%; flow rate 1.8 mL/min). Anal. ($C_{31}H_{37}NO_{10}S$·HCl) C, H, Cl, N, S.

14-*n*-Pentyl-14-thiaadriamycin (9). 1-Pentanethiol (60 μL, 0.57 mmol) was added to a stirred suspension of 6 (300 mg, 0.467 mmol) in MeOH (30 mL) and anhydrous K_2CO_3 (75 mg) at room temperature, and the reaction mixture was stirred for 30 min, then diluted with $CHCl_3$ (150 mL), and washed with pH 10 buffer (2 × 50 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and filtered, and the solvent was removed by distillation. The residue was chromatographed on deactivated BioSil (15 g). $CHCl_3$–MeOH (3%) eluted pure 9, from which the hydrochloride was made as before (150 mg, 54.3%): homogeneous on TLC (B; R_f 0.71) and HPLC (t_R = 5.05 min; CH_3CN, 55%/pH 4 buffer, 45%; flow rate 1.0 mL/min). Anal. ($C_{32}H_{39}NO_{10}S$·HCl·H_2O) C, H, Cl, N, S.

14-*n*-Dodecyl-14-thiaadriamycin (10). 1-Dodecanethiol (250 μL, 1.05 mmol) was added with stirring to a suspension of 6 (300 mg, 0.467 mmol) in MeOH (30 mL) and anhydrous K_2CO_3 (75 mg) at room temperature. The reaction was complete after 30 min and was worked up as above. The crude product was chromatographed on deactivated BioSil (15 g). $CHCl_3$–MeOH (3%) eluted pure 10 (155 mg, 53.3%): homogeneous on TLC (B; R_f 0.80) and HPLC (t_R = 4.22 min; CH_3CN, 70%/pH 4 buffer, 30%; flow rate 1.2 mL/min). Anal. ($C_{39}H_{53}NO_{10}S$) C, H, N, S.

14-Phenyl-14-thiaadriamycin (11). To a solution of 6 (300 mg, 0.467 mmol) in MeOH (30 mL) was added, in succession, anhydrous K_2CO_3 (75 mg) and benzenethiol (50 μL, 0.49 mmol) at room temperature, and the mixture was stirred for 30 min. The reaction product, worked up as before, was chromatographed on deactivated BioSil (15 g). $CHCl_3$–MeOH (4%) eluted pure 11 (142 mg, 52.7%): homogeneous on TLC (B; R_f 0.69) and HPLC (t_R = 4.29 min; CH_3CN, 55%/pH 4 buffer 45% flow rate 1.0 mL/min). Anal. ($C_{33}H_{33}NO_{10}S$·0.5H_2O) C, H, N, S.

14-(Carbethoxymethyl)-14-thiaadriamycin (12). To a suspension of 6 (200 mg, 0.311 mmol) in MeOH (20 mL) and anhydrous K_2CO_3 (50 mg) was added ethyl mercaptoacetate (40 μL, 0.365 mmol), and the reaction mixture was stirred at room temperature for 30 min. The product was worked up as before and chromatographed on deactivated BioSil (15 g). $CHCl_3$–MeOH (5%) eluted pure 12, from which the hydrochloride was made with 0.1 N HCl (142 mg, 66.9%): homogeneous on TLC (B; R_f 0.64) and HPLC (t_R = 5.59 min; CH_3CN, 42%/pH 4 buffer, 58%; flow rate 1.0 mL/min). Anal. ($C_{31}H_{35}NO_{12}S$·HCl·0.5H_2O) C, H, N; S: calcd, 4.64; found, 4.21.

***N*-(Trifluoroacetyl)-14-bromodaunorubicin (7).** To 6 (2 g, 3.11 mmol) suspended in ethyl acetate (200 ml) and stirred at room temperature was added all at once trifluoroacetic anhydride (3.5 mL) to give a clear solution. The reaction, complete almost instantaneously (TLC, B), was allowed to run for 10 min. The mixture was diluted with $CHCl_3$ (500 mL) and washed with water (2 × 200 mL) and pH 7 buffer (1 × 100 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and filtered, and the filtrate was evaporated to dryness. The residue was refluxed in MeOH (100 ml) for 10 min, evaporated to dryness, and precipitated from $CHCl_3$–petroleum ether. The product showed the presence of TFA ester ($OCOCF_3$; IR 1790 cm^{-1}). The partially purified material was further refluxed twice for 10-min periods with MeOH (50 mL each time) and reprecipitated from $CHCl_3$–petroleum ether to yield the TFA ester-free amide, contaminated with small amounts of other impurities (1.6 g). This material was chromatographed on BioSil A (100 g). $CHCl_3$ eluted pure 7 (680 mg, 31%), homogeneous on TLC (A, R_f 0.69) and HPLC (t_R = 3.47 min; CH_3CN, 42%/pH 4 buffer, 58%; flow rate 1.0 mL/min).

***N*-(Trifluoroacetyl)-14-*n*-butyl-14-thiaadriamycin (13).** To a solution of 7 (250 mg, 0.334 mmol) in absolute ethanol (50 mL) was added, with stirring at room temperature, anhydrous K_2CO_3 (100 mg, 0.736 mmol) and 1-butanethiol (35 μL, 0.39 mmol). A red precipitate separated immediately. The progress of the reaction was followed by TLC (A). The reaction was complete after 30 min. The reaction mixture was diluted with $CHCl_3$ (250 mL) and washed with pH 7 buffer (2 × 75 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and filtered, and the solvent was removed on the rotary evaporator. The residue was precipitated from $CHCl_3$–petroleum ether to yield the product 13 (232 mg, 89%): homogeneous on TLC (A; R_f 0.86) and HPLC (t_R = 2.75 min; CH_3CN, 50%/pH 4 buffer, 50%; flow rate 1.2 mL/min). Anal. ($C_{33}H_{36}F_3NO_{11}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)-14-*n*-pentyl-14-thiaadriamycin (14).** *N*-(Trifluoroacetyl)-14-bromodaunorubicin (7; 250 mg, 0.334 mmol) in absolute ethanol (50 mL) and anhydrous K_2CO_3 (100 mg) was stirred at room temperature, and 1-pentanethiol (50 μL, 0.4 mmol) was added. The reaction was complete after 30 min. The product was worked up as before and precipitated from $CHCl_3$–petroleum ether to yield pure 14 (230 mg, 89%): homogeneous on TLC (A; R_f 0.83) and HPLC (t_R = 2.97 min; CH_3CN, 50%/pH 4 buffer, 50%; flow rate 1.2 mL/min). Anal. ($C_{34}H_{38}F_3NO_{11}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)-14-*n*-dodecyl-14-thiaadriamycin (15).** 1-Dodecanethiol (90 μL, 0.393 mmol) was added at room temperature to a solution of 7 (250 mg, 0.334 mmol) in absolute ethanol (50 mL) containing anhydrous K_2CO_3 (100 mg). The reaction product was worked up after 30 min, as before, and precipitated from $CHCl_3$–petroleum ether to obtain the product, 15 (250 mg; 91%): homogeneous on TLC (A, R_f 0.85) and HPLC (t_R = 3.04 min; CH_3CN, 62%/pH 4 buffer, 38%; flow rate 1.4

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Table III

no.	reaction time, min	amount of dithiol,[a] μL	yield, %	formula	anal.
21	20	30 + 8 + 5	36	$C_{57}H_{62}N_2O_{20}S_2 \cdot H_2O$	C, H, N, S
22	30	30 + 10 + 5	34	$C_{60}H_{68}N_2O_{20}S_2 \cdot H_2O$	C, H, N, S
23	20	30 + 10 + 10	42	$C_{63}H_{74}N_2O_{20}S_2 \cdot H_2O$	C, H, N, S

[a] ~4×10^{-4} mol.

mL/min). Anal. ($C_{41}H_{52}F_3NO_{11}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)-14-phenyl-14-thiaadriamycin (16).** To a stirred solution of **7** (250 mg, 0.334 mmol) in absolute ethanol (50 mL) was added, in succession, anhydrous K_2CO_3 (100 mg) and benzenethiol (40 μL, 0.40 mmol). The reaction was run for 30 min, and the product was worked up as before. Precipitation from $CHCl_3$–petroleum ether gave pure **16** (243 mg, 93%): homogeneous on TLC (A, R_f 0.77) and HPLC (t_R = 4.29 min; CH_3CN, 45%/pH 4 buffer, 55%; flow rate 1.2 mL/min). Anal. ($C_{35}H_{32}F_3NO_{11}S$) C, H, F, N, S.

***N*-(Trifluoroacetyl)-14-(carbethoxymethyl)-14-thiaadriamycin (17).** *N*-(Trifluoroacetyl)-14-bromodaunorubicin (**7**; 250 mg, 0.33 mmol) was stirred at room temperature in absolute ethanol (50 mL). Anhydrous K_2CO_3 (100 mg) was added, followed by ethyl mercaptoacetate (43 μL, 0.40 mmol). The reaction mixture was stirred for 30 min. The product was worked up as before and precipitated from $CHCl_3$–petroleum ether to obtain pure **17** (248 mg, 93.9%): homogeneous on TLC (A, R_f 0.70) and HPLC (t_R = 4.26 min; CH_3CN, 42%/pH 4 buffer, 58%; flow rate 1.0 mL/min). Anal. ($C_{33}H_{34}F_3NO_{13}S \cdot 0.5H_2O$) C, H, F, N, S.

14-Phenyl-14-selenaadriamycin (18). To 14-bromodaunorubicin (**6**; 250 mg, 0.389 mmol) in methanol (25 mL) and anhydrous K_2CO_3 (125 mg) was added benzeneselenol (64 mg, 0.40 mmol) in three small portions at 2-min intervals. The reaction was stirred for 10 min. $CHCl_3$ (50 mL) was added to the reaction mixture and then pH 10 buffer (15 mL). The organic layer was washed once more with pH 10 buffer (15 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on BioSil A. Elution with $CHCl_3$–MeOH (5%) afforded pure **18** (110 mg, 41%), homogeneous on TLC (B) and HPLC (t_R = 5.87 min; CH_3CN, 35 to 62% linear gradient over 6 min, with pH 4.0 buffer; flow rate 3.5 mL/min). The hydrochloride salt was prepared as usual (116 mg). Anal. ($C_{33}H_{34}NO_{10}Se \cdot HCl \cdot 1.5H_2O$) C, H, N, Se.

14-(Carbethoxymethyl)-1-thiaadriamycinone. 14-Bromodaunomycinone[22] (50 mg, 0.105 mmol) in absolute ethanol (15 mL), anhydrous K_2CO_3 (25 mg), and ethyl mercaptoacetate (15 mL, 0.137 mmol) were heated at reflux for 5 min. The reaction mixture was cooled and concentrated to dryness. The mixture was taken up in $CHCl_3$ (30 mL) and pH 10 buffer (15 mL). The $CHCl_3$ layer was then washed with pH 7 buffer (15 mL), dried (Na_2SO_4), and evaporated to dryness on the rotary evaporator. The residue was crystallized from petroleum ether–EtOAc to give pure product (41 mg, 76%). Anal. ($C_{25}H_{24}O_{10}S$) C, H; S: calcd, 6.21; found, 5.65.

14-Phenyl-14-thiaadriamycinone. 14-Bromodaunomycinone (50 mg, 0.105 mmol) in absolute ethanol (15 mL), anhydrous K_2CO_3 (25 mg), and benzenethiol (12.5 μL, 0.122 mmol) were heated at reflux for 5 min. The reaction mixture was evaporated to dryness on the rotary evaporator, and the residue was treated with a mixture of $CHCl_3$ (30 mL) and pH 10 buffer (20 mL). The $CHCl_3$ layer was washed with pH 7 buffer (20 mL), dried (Na_2SO_4), and concentrated to dryness. The mixture was filtered from $CHCl_3$–petroleum ether to obtain the product (45 mg, 94%). Anal. ($C_{27}H_{22}O_8S$) C, H, S.

14-*n*-Dodecyl-14-thiaadriamycinone. 14-Bromodaunomycinone (50 mg, 0.105 mmol) was suspended in absolute ethanol (15 mL). Anhydrous K_2CO_3 (25 mg) was added, followed immediately by dodecanethiol (27 μL, 0.112 mmol). The reaction mixture was heated at reflux for 10 min, at which time it became a clear red solution. The mixture was cooled, and the solvent was evaporated to dryness on the rotary evaporator. The residue was dissolved in $CHCl_3$ (30 mL) and pH 10 buffer (20 mL). The $CHCl_3$ layer was washed with pH 7 buffer (20 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from ethanol to yield the product in high purity (48 mg, 76%). Anal. ($C_{33}H_{42}O_8S$) C, H, S.

Preparation of Bis(thiaadriamycin)s. *S,S*-Bis(daunorubicin-14-yl)ethane-1,2-dithiol (20). 14-Bromodaunorubicin (**6**; 500 mg, 0.78 mmol) was stirred at room temperature in MeOH (50 mL) containing anhydrous K_2CO_3 (200 mg). Ethane-1,2-dithiol (43 μL, 0.40 mmol) was added in three portions (30 μL plus 8 μL after 5 min plus 5 μL after another 5 min). The course of the reaction was followed by TLC (B). At the end of stirring for an overall 20 min, no starting material was discernible. The reaction mixture was diluted with $CHCl_3$ (200 mL) and washed with pH 10 buffer (2 × 50 mL). The $CHCl_3$ extract was dried (Na_2SO_4), the solvent was removed, and the residue was precipitated from $CHCl_3$–petroleum ether (0.365 g). This material was chromatographed on BioSil A (washed and reactivated, 15 g). $CHCl_3$–MeOH (15%) eluted pure **20** (0.160 g, 33.3%): homogeneous by TLC. Anal. ($C_{56}H_{60}N_2O_{20}S_2 \cdot 2H_2O$) C, H, N, S.

Other bis(thiaadriamycin)s were made essentially by this same procedure, except for the differences noted in Table III.

Acknowledgment. This work was supported by Research Grant CA 17263 and National Research Service Award CA 05130 (to W.J.P.) from the National Cancer Institute, National Institutes of Health, Bethesda, MD. The authors are grateful to Adria Laboratories Inc., Columbus, OH, and Farmitalia, Milan, Italy, for providing the generous supply of daunorubicin used in this work.

Registry No. **2**, 23214-92-8; **6**, 65026-79-1; **7**, 77270-18-9; **8**, 83291-64-9; **8**·HCl, 83349-71-7; **9**, 83291-65-0; **9**·HCl, 83349-72-8; **10**, 83291-66-1; **11**, 83291-67-2; **12**, 83291-68-3; **12**·HCl, 83349-73-9; **13**, 83311-77-7; **14**, 83291-69-4; **15**, 83291-70-7; **16**, 77270-20-3; **17**, 83291-71-8; **18**, 83291-72-9; **18**·HCl, 83349-74-0; **19**, 77270-19-0; **20**, 83291-73-0; **21**, 83291-74-1; **22**, 83291-75-2; **23**, 83291-76-3; 14-(carbethoxymethyl)-14-thiaadriamycinone, 83291-77-4; 14-phenyl-14-thiaadriamycinone, 83291-78-5; 14-dodecyl-14-thiaadriamycinone, 83291-79-6; 1-butanethiol, 109-79-5; 1-pentanethiol, 110-66-7; 1-dodecanethiol, 112-55-0; benzenethiol, 108-98-5; ethyl mercaptoacetate, 623-51-8; benzeneselenol, 645-96-5; 14-bromodaunomycinone, 29742-69-6; ethane-1,2-dithiol, 540-63-6.