(B) 0.30; reverse-phase HPLC retention time 10.0 min (compared to **2**, 7.8 min; daunomycinone, 3.6 min; **9**, 14.4 min).

A small sample was converted back to free base **12** by dissolving it in H_2O, making the solution basic with $NaHCO_3$ solution, and then extracting it with $CHCl_3$. The $CHCl_3$ solution was dried, concentrated, and used to obtain an NMR spectrum: NMR (360 MHz; $CDCl_3$) δ 13.975 and 13.968 (2 s, 6-OH), 13.319 and 13.309 (2 s, 11-OH), 8.021 (d, 1-H, $J_{1,2}$ = 7.55 Hz), 7.777 (t, 2-H), 7.385 (d, 3-H, $J_{2,3}$ = 8.45 Hz), 5.561 (d, 1′-H, J = 2.99 Hz), 5.29 (br s, 7-H), 4.081 (s, 4-OMe), 4.0–4.1 (m, 5′-H), 3.67 [m, morpholino $(CH_2)_2O$, 3′-H, 4′-H), 3.20 (d, 10-H_B, J_{gem} = 20 Hz), 2.97 (br s, OH), 2.25–2.75 [m, morpholino $(CH_2)_2N$, 13-H, 10-H_A, (OH, OH)], 1.65–1.95 (m, 2′-H_2, 8-H_2), 1.39 (d, 6′-H_3, J = 6.53 Hz), 1.336 and 1.292 (2 d, 14-H_3, J = 6.4 Hz).

1,2-Dihydroxy-4-methoxycyclopentane (19). 4-Methoxycyclopentene[10] (**18**) was converted[11] to **19**. The light yellow syrup, obtained in 47% yield by repeated ether extraction of the reaction mixture diluted with $NaHSO_3$ (20% solution) and saturated with NaCl, showed a trace impurity on TLC but was suitable for use in the next step: TLC (B) 0.4; NMR ($CDCl_3$) δ 3.8–4.3 (m, 1-H, 2-H, 4-H), 3.60 (s, 1-OH, 2-OH), 3.28 (s, OMe), 1.8–2.0 (m, 3-H_2, 4-H_2).

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Adriamycin Analogues. Preparation and Antitumor Evaluation of 7-*O*-(β-D-Glucosaminyl)daunomycinone and 7-*O*-(β-D-Glucosaminyl)adriamycinone and Their *N*-Trifluoroacetyl Derivatives

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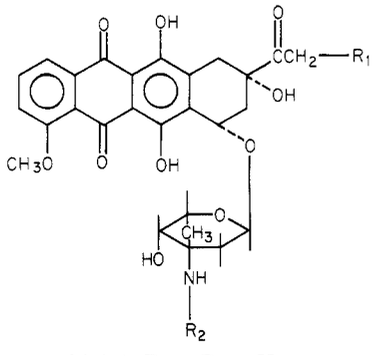
The title compounds were prepared by Koenigs–Knorr condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-α-D-glucopyranosyl bromide with daunomycinone or a side-chain protected adriamycinone, followed by selective hydrolysis of blocking groups. Despite poor complexation with DNA and weak growth-inhibitory properties in vitro, the glucosaminyl analogues of the antitumor antibiotics daunorubicin and adriamycin, at their optimal (highest nontoxic) doses, exhibited antileukemic activity equivalent to that of adriamycin against a usually drug-refractory mouse leukemia model system (L1210) in vivo. These findings, together with other data from these laboratories, continue to support the hypothesis that the mechanism of action of adriamycin and related agents cannot be due exclusively to DNA binding, as has earlier been believed.

The antitumor antibiotics daunorubicin (**1a**) and adriamycin (**1b**) are clinically important cancer chemotherapeutic agents. Adriamycin occupies a special place in cancer medicine because of its effectiveness against a range of solid tumors normally refractory to drug treatment.[1-3] These agents are known to bind strongly to DNA, and this biological property has generally been accepted as their mechanism of antitumor action.[3,4]

In connection with a major program on the chemistry, biology, and pharmacology of anthracyclines, these laboratories have been responsible for the synthesis, preclinical development, and clinical introduction of the promising adriamycin analogue *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32, **1c**).[5,6] In contrast to the natural occurring agents **1a** and **1b**, the semisynthetic derivative **1c** does not bind to double-helical calf thymus DNA.[7,8] However, **1c**



1a (daunorubicin), $R_1 = R_2 = H$
b (adriamycin), $R_1 = OH$; $R_2 = H$
c (AD 32), $R_1 = OCO(CH_2)_3CH_3$; $R_2 = COCF_3$

exhibits very significant in vivo antitumor activity: therapeutic superiority to adriamycin has been seen across a spectrum of experimental murine tumor systems, including early and advanced leukemias, solid tumors, and a leukemia subline selected for adriamycin resistance.[5,9,10] Clinical antitumor activity, and low toxicity relative to

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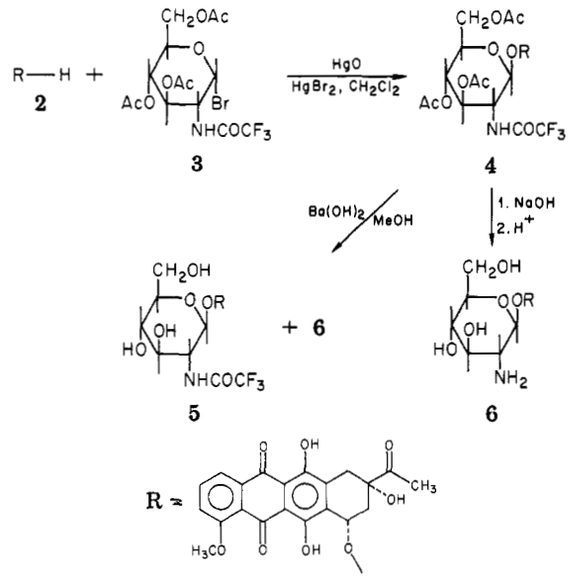
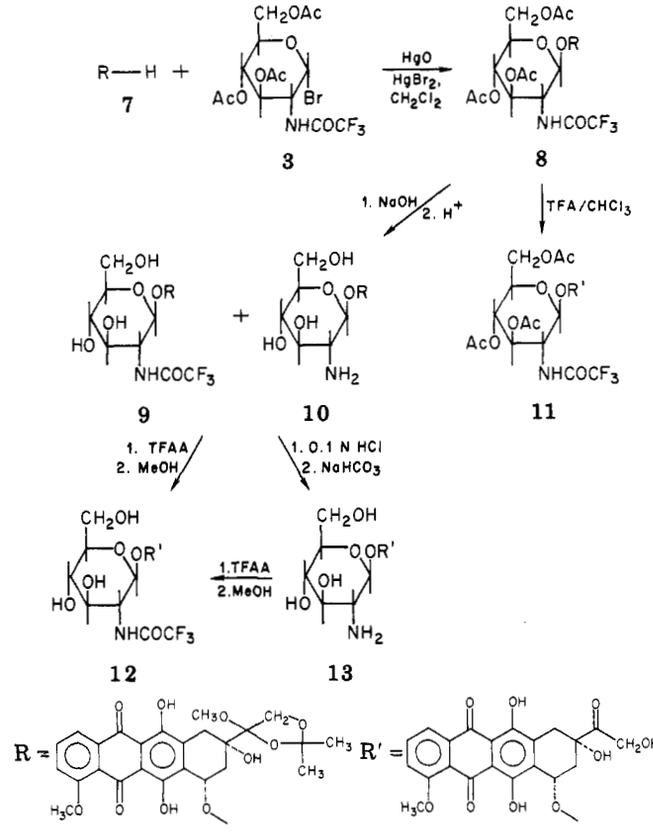
Scheme I



Scheme II



adriamycin, have been documented for AD 32 in connection with phase I/II trials.[11,12] The pharmacology of AD 32 has been studied extensively in laboratory animals and humans; considerable evidence has been gathered to show that **1c** does not give rise to significant quantities of adriamycin, nor is it apparently metabolized to any other DNA-binding species.[13-18]

Thus, while the mechanism of action of **1c** remains speculative, the potent activity of this non-DNA binding analogue raises questions about the validity of the DNA-binding hypothesis for the parent antibiotics. We have been interested in examining this question, in part, by a structure–activity analysis approach. The present report concerns the synthesis and biological evaluation of some analogues of **1a** and **1b**, in which the naturally occurring 3-amino sugar, daunosamine, has been replaced by a 2-aminopyranoside. 7-*O*-(β-D-Glucosaminyl)daunomycinone (**6**) was actually first prepared by Penco in 1968.[19] At the time, its lack of DNA binding and its poor in vitro growth-inhibitory activity were used in partial support of the mechanistic hypothesis requiring an unsubstituted amino function at position 3 of the glycoside for DNA complexation and antitumor activity.[4] We decided to reinvestigate the biological properties of **6** and, in view of the activity of **1c**, to also examine the in vitro and in vivo properties of **5**, the trifluoroacetamido derivative of **6**. Furthermore, because of the better therapeutic index of **1b** compared to **1a**, we have also prepared the hitherto unknown corresponding adriamycin analogues **13** and **12** for inclusion in the study.

Chemistry. The preparation of 7-*O*-(β-D-glucosaminyl)daunomycinone (**6**) and 7-*O*-(β-D-glucosaminyl)adriamycinone (**13**) and their derivatives were carried out as outlined in Schemes I and II, respectively. Condensation of daunomycinone (**2**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-α-D-glucopyranosyl bromide (**3**)[20] under Koenigs–Knorr conditions gave as the major product the fully protected glycoside **4** in 60% yield after chromatography. Hydrolysis of **4** with $Ba(OH)_2$ in MeOH overnight at 0 °C gave the partially deprotected glycoside **5** in 63% yield plus a 17% yield of the fully deprotected glycoside **6**. Alternatively, treatment of **4** with 0.1 N NaOH for 30 min at room temperature, followed by neutralization, gave compound **6** in 70% yield.

In a like manner, treatment of the protected adriamycinone derivative **7**[21] with the bromo sugar **3** gave the coupled product **8** in 69% yield after chromatography. Subsequent alkaline hydrolysis of **8** with 0.1 N NaOH gave a 1:1 mixture of the partially deblocked and fully deblocked glycosides **9** and **10**, respectively (compound **9** showed typical amide absorption at 1700 cm^{-1} and **10** showed no such absorption). As expected, no evidence of hydrolysis of the isopropylidene groupings was observed (IR and NMR) under these alkaline conditions. Mild acid treatment ($TFA/CHCl_3$), on the other hand, caused complete hydrolysis of the isopropylidene protecting group of **8** but left the sugar amide and ester linkages intact, thereby

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affording in good yield the fully carbohydrate-protected adriamycinone derivative **11**. 7-*O*-(β-D-Glucosaminyl)-adriamycinone (**13**) was obtained by mild acid hydrolysis of the **9**–**10** mixture, followed by neutralization. The corresponding trifluoroacetamide **12** was prepared from **13** by treatment with trifluoroacetic anhydride, followed by methanolysis; **12** could be obtained alternatively from the **9**–**10** mixture by treatment with trifluoroacetic anhydride, a procedure which hydrolyzed the isopropylidene protecting groups and trifluoroacetylated the unsubstituted amino function of **10** in a single step.

In all compounds the configuration at the anomeric carbon has been assigned as β based on the general course of the Koenigs–Knorr glycosidation reaction,[22] in which the 1,2-trans glycoside is formed preferentially, and on the fact that reaction of **3** with other alcohols has been found to yield only the β isomer.[23] This has also been claimed for the coupling product prepared by Penco.[19] Unfortunately, attempts to substantiate this claim by NMR have been unsuccessful. Although the α and β anomers of 2-aminoglucopyranoses are in general quite easily assignable by determination of the coupling constants of the anomeric proton,[24] with the α isomer having a coupling constant of 4 Hz and the β isomer one of 8 Hz, in compound **4** the C-7 proton, the anomeric C-1′ proton, and an additional carbohydrate proton absorb in the same region (δ 5.0–5.2); thus, determination of the coupling constant of C-1′ has not been possible.

With regard to the stereospecificity of the coupling reaction, careful chromatography of the product resulting from the reaction of **2** with **3** yielded, in addition to **4**, a product of slightly lower R_f (yield 0.15%), which gave a nearly superimposable IR spectrum with that of **4**. This product is believed to be the α isomer of **4**. It is likely that the α isomer of **8** is also formed in the reaction of **7** with **3**, although this has not been rigorously proven. However, a faint spot of slightly lower R_f than **8** is observable on TLC of the more polar eluents from the column employed for the purification of **8**.

Biology. Drug interaction with calf-thymus DNA was measured spectrophotometrically[25-27] at 480 nm in pH 7.0 buffer by taking the ratio of the absorbance of a drug–DNA mixture (A) relative to that of drug in the absence of DNA (A_0). Thus, the smaller the A/A_0 value is from unity, the greater is the degree of drug–DNA complexation. By this method, adriamycin (**1b**) exhibits an A/A_0 value of 0.60. In contrast, **6** and **13** had A/A_0 values in the range of 0.85, indicating some, but relatively little, DNA interaction. Furthermore, and in contrast to adriamycin, **6** and **13** are easily freed from the DNA by simple washing (ultrafiltration). A/A_0 values for the trifluoroacetamides **5** and **12** were not determined, since these compounds were not sufficiently soluble under the experimental conditions and, besides, they would not be expected to show any binding properties.

Compounds **4**–**6** and **8**–**13** were assayed for growth-inhibitory activity against CCRF-CEM (human lymphoblastic leukemic) cells in culture according to a previously described procedure.[28] In this system, adriamycin is strongly growth inhibitory, a concentration of 0.066 μM producing 50% inhibition of growth (ID_{50} value). Of the nine compounds evaluated, only **6** (ID_{50} = 3.0 μM) and **9** (ID_{50} = 1.61 μM) were active at concentrations below 5.0 μg/mL. In general, however, it is the experience of this laboratory with adriamycin analogues that in vitro ID_{50} values do not predict for in vivo antitumor activity but rather serve as a measure of toxicity, thereby permitting a reasonable selection of dosage levels a priori for in vivo assays.

Table I. Activity of 7-*O*-(β-D-Glucosaminyl)daunomycinone and 7-*O*-(β-D-Glycosaminyl)adriamycinone against L1210 Murine Leukemia in Vivo[a]

compd	optimal dose, (mg/kg)/day	median day of death	increase in life span, %
controls		10.0	
adriamycin (**1b**)	3.5	14.0	+40
6	90	16.0	+60
13	120	14.0	+40

[a] BDF_1 male mice; tumor inoculum 10^5 cells ip; treatment daily on days 1–4 ip.

The murine L1210 leukemia system[29] was used for the antitumor evaluation of compounds **4**–**6** and **11**–**13**. The agents, as well as adriamycin as a positive control, were each run through a dose–response range, with therapy starting on the 1st day after tumor implantation and continuing daily for 4 days. Adriamycin was given dissolved in saline, and the other agents were dissolved (**6** and **13**) or suspended in 10% aqueous Tween 80 (Polysorbate 80; Atlas Chemical Co., Wilmington, DE) vehicle.[30] Compounds **4**, **5**, **11**, and **12** were inactive in this system up to the highest dosages used (120, 120, 100, and 100 mg/kg, respectively). However, as seen in Table I, **13** was as active as adriamycin and **6** was somewhat more effective than adriamycin in prolonging the survival of tumor-bearing animals. The antitumor activity of **6** was previously unrecognized because earlier workers failed to achieve an optimal dose.[4,19] Since neither **6** nor **13** has a 3-amino glycosidic function and neither complexes significantly with DNA, we believe the observed antitumor activity of these compounds implicates the chromophore as being primarily responsible for the antitumor effects exhibited by these and other anthracyclines. This work has led us to examine other anthracycline analogues with fraudulent glycosides in the search for adriamycin analogues with improved antitumor activity and/or reduced toxicity relative to adriamycin.

Experimental Section

Melting points were taken on a Kofler hot stage with microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 137B Infracord. Proton NMR were recorded on a Varian T60A System spectrometer with Me_4Si as internal standard; but resolution of many of the glycosidic protons, especially the anomeric protons, was inadequate (vide supra). Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6E double-focusing spectrometer, operated at 70-eV ionizing energy. Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN, and are within ±0.4%, unless otherwise noted.

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TLC on silica gel G (Analtech), using either $CHCl_3$–MeOH (49:1; system A) or $CHCl_3$–MeOH–H_2O (120:20:1; system B) as irrigant, was used for identification and for evaluation of homogeneity. Column chromatography was carried out on BioSil A silicic acid (100–200 mesh, Bio-Rad).

3,4,6-Tri-O-acetyl-2-[(trifluoroacetyl)amino]-α-D-glucopyranosyl bromide (2) was prepared in 72% yield by treatment of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucopyranose[20] with HBr/HOAc, mp 95–97 °C (lit.[20] mp 96 °C).

7-O-[3,4,6-Tri-O-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyl]daunomycinone (4). To 0.500 g (1.24 mmol) of daunomycinone in 60 mL of anhydrous CH_2Cl_2 were added successively, with stirring, 3.0 g of type 3Å molecular sieves, 1.50 g (6.93 mmol) of HgO, 0.375 g (1.04 mmol) of $HgBr_2$, and 0.800 g (1.72 mmol) of bromo sugar **3**. The reaction mixture was then allowed to stir at room temperature in the dark (foil-covered flask), with periodic monitoring by TLC (solvent system A). After 1.5 and 2.5 h, an additional 0.800 g (1.72 mmol) and 0.500 g (1.11 mmol) of **3** were added. After a total reaction time of 4 h, the mixture was filtered, and the filtrate was evaporated to dryness. The semisolid residue was crystallized from $CHCl_3$–petroleum ether to give 0.987 g of an amorphous red-orange powder, shown by TLC (A) to contain only coupled products and carbohydrate. Subsequent chromatography, $CHCl_3$ elution, afforded 0.650 g of pure **4**. Crystallization from $CHCl_3$–petroleum ether yielded 0.595 g (60.7%) of **4** as a light orange amorphous powder: mp 275–278 °C dec (lit.[19] mp 282–295 °C); mass spectrum, *m/e* 781 (M^+); NMR ($CDCl_3$) δ 2.03, 2.08, and 2.12 (3 s, 3 H each, $OCOCH_3$), 2.43 (s, 3 H, 9-$COCH_3$), 4.05 (s, 3 H, C_4 OCH_3), 5.17 (m, 3 H, C_7 H, $C_{1'}$ H, and other), 6.77 (d, J = 8 Hz, 1 H, $NHCOCF_3$), 7.30–7.97 (m, 3 H, aromatic), 13.14 and 13.86 (2 s, 2 H, phenolic OH); TLC (solvent system A), single spot, R_f 0.18. Anal. ($C_{35}H_{34}F_3NO_{16}$) C, H, F, N.

Further elution of the above column with $CHCl_3$–EtOAc (8:2, v/v) afforded 10.0 mg of a mixture containing compound **4** and material of slightly lower R_f, 0.17. Purification of this mixture on two additional columns afforded 1.5 mg (0.15%) of pure material, assumed to be **4** α anomer: TLC (solvent system A), single spot, R_f 0.17.

7-O-[2-Deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyl]-daunomycinone (5). To 0.91 g (0.245 mmol) of **4** was added, with ice cooling, an ice-cold solution of $Ba(OH)_2 \cdot 8H_2O$ in MeOH (16 mL of a 10 g/100 mL solution). The resulting violet solution was stoppered and allowed to stand undisturbed at 0 °C for 16 h. The solution was then treated with CO_2 for 5 min, 16 mL of MeOH was added, and the precipitated barium salts were filtered off. Evaporation of the filtrate yielded a semisolid, which was immediately chromatographed. Elution with solvent system B afforded 0.102 g (63.8%) of **5** and 0.024 g (17.5%) of **6**. Recrystallization of **5** from $CHCl_3$–MeOH–petroleum ether gave 0.095 g (60.2%) of an orange-red amorphous powder: mp 235–238 °C dec; TLC (solvent system B), single spot, R_f 0.29. Anal. ($C_{29}H_{28}F_3NO_{13} \cdot H_2O$) C, H, N; F: calcd, 8.46; found, 7.92.

7-O-(2-Deoxy-2-amino-β-D-glucosyl)daunomycinone (6). To 200 mg (0.258 mmol) of **4** dissolved in 16 mL of acetone and stirred under N_2 was added, all at once, 80 mL of 0.1 N NaOH. After stirring for 30 min at room temperature, the reaction mixture was cooled in an ice–water bath, and the pH was adjusted to 8.4 by the dropwise addition of 0.1 N HCl. The solution was then extracted with $CHCl_3$ (2 × 200 mL) to remove unreacted starting material and then repeatedly with $CHCl_3$–MeOH (8:2). The alcoholic extracts were then dried (Na_2SO_4) and evaporated to yield the free base **6** as a red-orange solid. The hydrochloride of **6** was obtained by dissolving the free base in $CHCl_3$–MeOH (1:1), followed by the addition of the stoichiometric amount of methanolic HCl and ether: yield 0.120 g (78.7%); mp 220 °C dec; TLC (solvent system B), single spot, R_f 0.18. Anal. ($C_{27}H_{29}NO_{12} \cdot HCl \cdot CHCl_3 \cdot H_2O$) C, H, N.

9-Deacetyl-9-(2,2-dimethyl-4-methoxydioxolan-4-yl)daunomycinone (7) was prepared in 51.6% yield by treating adriamycinone[31] with 2,2-dimethoxypropane in the presence of TsOH, according to the procedure of Arcamone et al.[21]

7-O-[3,4,6-Tri-O-acetyl-2-deoxy-2-[(trifluoroacetyl)-amino]-β-D-glucosyl]-9-deacetyl-9-(2,2-dimethyl-4-methoxydioxolan-4-yl)daunomycinone (8). To 0.549 g (1.28 mmol) of **7** dissolved in 40 mL of CH_2Cl_2 were added successively 3.0 g of type 3Å molecular sieves, 1.05 g (4.85 mmol) of HgO, 0.270 g (0.75 mmol) of $HgBr_2$, and 0.75 g (1.62 mmol) of bromo sugar **3**. The reaction mixture was then allowed to stir at room temperature in the dark (foil covered flask), with periodic monitoring by TLC (solvent system A). After 1 h, an additional 0.75 g (1.62 mmol) of **3** was added. After a total reaction time of 2.5 h, the mixture was filtered, and the filtrate was evaporated to dryness. The semisolid residue was crystallized from $CHCl_3$–petroleum ether to give 0.870 g of an orange-red amorphous powder, shown by TLC (A) to contain mostly (>90%) **8**, contaminated only with unreacted starting material. Subsequent chromatography, eluent $CHCl_3$, followed by crystallization from $CHCl_3$–petroleum ether, gave 0.618 g (69.4%) of **8** as a red amorphous powder: mp 240–242 °C dec; NMR ($CDCl_3$) δ 1.47 and 1.65 (2 s, 3 H each, dioxolane CH_3), 2.02, 2.07, and 2.13 (3 s, 3 H each, $OCOCH_3$), 3.42 (s, 3 H, C_{13} OCH_3), 4.08 (s, 3 H, C_4 OCH_3), 5.18 (br m, 3 H, C_7 H, $C_{1'}$ H, and other), 7.30–8.14 (3 H, aromatic), 13.13 and 13.91 (2 s, 2 H, phenolic OH); TLC (solvent system A), single spot, R_f 0.25; TLC (solvent system B), single spot, R_f 0.91. Anal. ($C_{39}H_{42}F_3NO_{18}$) C, H, F, N.

7-O-[3,4,6-Tri-O-acetyl-2-deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyl]adriamycinone (11). To 0.170 g (0.196 mmol) of **8**, dissolved in 150 mL of $CHCl_3$, was added 1.0 mL of TFA, and the resulting solution was warmed on a steam bath for 10 min. An additional 1.0 mL of TFA was added, and the solution was warmed for a further 5 min. The reaction mixture was then cooled, 50 mL of H_2O was added, and the organic phase was separated and washed with an additional 100 mL of H_2O. It was then dried (Na_2SO_4) and evaporated to give 0.143 g of an orange solid. Recrystallization from $CHCl_3$–petroleum ether afforded 0.132 g (85.2%) of **11** as an orange amorphous powder: mp 269–270 °C dec; TLC (solvent system B), single spot, R_f 0.75. Anal. ($C_{35}H_{34}F_3NO_{17}$) C, H, F, N.

7-O-[2-Deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyl]-9-deacetyl-9-(2,2-dimethyl-4-methoxydioxolan-4-yl)daunomycinone (9) and 7-O-(2-Deoxy-2-amino-β-D-glucosyl)-9-deacetyl-9-(2,2-dimethyl-4-methoxydioxolan-4-yl)daunomycinone (10). To 0.300 g (0.345 mmol) of **8** dissolved in 25 mL of acetone and stirred under N_2 was added 100 mL of 0.1 N NaOH. After 30 min at room temperature, the pH was adjusted to 8.4 by the dropwise addition of 0.1 N HCl, and the solution was repeatedly extracted with $CHCl_3$. The $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to give 0.200 g of a solid: TLC (solvent system B), two spots of equal intensity, R_f 0.35 and 0.28. Chromatography of a portion on silicic acid (solvent system B) gave the pure components, compounds **9** (R_f 0.35) and **10** (R_f 0.28).

7-O-[2-Deoxy-2-[(trifluoroacetyl)amino]-β-D-glucosyl]-adriamycinone (12). To 200 mg of the **9–10** mixture (from the preceding preparation) dissolved in 170 mL of an $CHCl_3$–ether mixture (1:1, v/v) and cooled in ice was added dropwise 17.0 mL of TFAA with stirring. After 10 min the solution was washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated to give a reddish-orange oil. The oil was dissolved in 50 mL of MeOH, heated at reflux for 20 min, cooled, and evaporated to give a solid. Recrystallization from $CHCl_3$–petroleum ether afforded 0.160 g (66.9%) of **12** as a rust-colored amorphous powder: mp 247–250 °C dec; TLC (solvent system B) single spot, R_f 0.17. Anal. ($C_{29}H_{28}F_3NO_{14}$) C, H, F, N.

7-O-(2-Deoxy-2-amino-β-D-glucosyl)adriamycinone (13). A solution of 0.080 g of the **9–10** mixture in 60 mL of 0.1 N HCl was allowed to stand undisturbed in the dark for 40 h. The reaction mixture was then extracted with $CHCl_3$ to remove trace aglycons and then neutralized by the addition of solid $NaHCO_3$ (0.50 g, 1 equiv). The solution was extracted with $CHCl_3$ (1 × 150 mL) and then $CHCl_3$–MeOH (7:3, v/v 4 × 200 mL). The alcoholic $CHCl_3$ extracts were dried and evaporated to give the free base as a solid. Treatment of a $CHCl_3$–MeOH (1:1) solution of the free base with methanolic HCl and ether afforded 0.049 g (60.2%) of the hydrochloride of **13** as a scarlet-red amorphous powder: mp 200 °C dec; TLC (solvent system B), single spot, R_f 0.10. Anal. ($C_{27}H_{29}NO_{13} \cdot HCl \cdot 3H_2O$) C, H, Cl, N.

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Adriamycin Analogues. Novel Anomeric Ribofuranoside Analogues of Daunorubicin[1]

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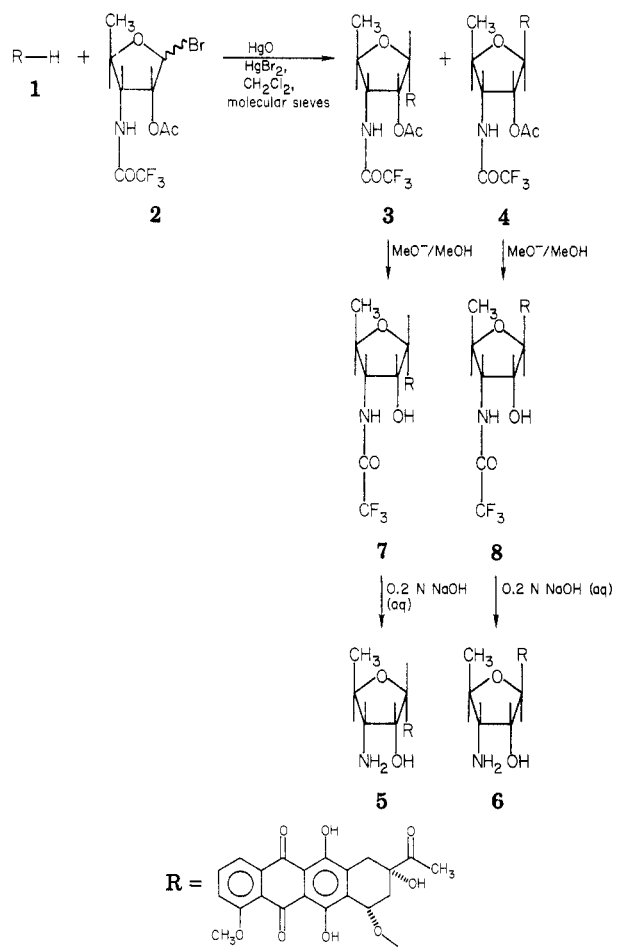
The synthesis, cell growth-inhibitory activity, in vivo antileukemic activity, and extent of DNA binding of the α- and β-anomeric 7-*O*-(3-amino-3,5-dideoxy-D-ribofuranosyl)daunomycinones and their trifluoroacetamides are described. These compounds are unique in that they are the first reported furanoside analogues of the antitumor antibiotics daunorubicin and adriamycin. Continuing analysis of structure–activity relationships amongst natural and semisynthetic anthracyclines fails to reveal a predictable relationship between in vivo antitumor activity and the in vitro properties of DNA complexation and cell growth inhibition.

This report concerns the synthesis and preliminary biological evaluation of the α and β anomers of 7-*O*-(3-amino-3,5-dideoxy-D-ribofuranosyl)daunomycinone and their trifluoroacetamide derivatives. These compounds are analogues of the anthracycline antitumor agents adriamycin, daunorubicin, and carminomycin and of AD 32 [*N*-(trifluoroacetyl)adriamycin 14-valerate],[2] an experimentally therapeutically superior and less toxic adriamycin analogue developed in these laboratories and currently in phase II clinical trial. The present aminofuranosides are unique in that, in so far as we are aware, no anthracycline analogue has yet been reported in which the naturally occurring 6-carbon daunosamine pyranose sugar has been replaced by a 5-carbon furanose moiety.

Adriamycin is one of the most important agents in cancer medicine today because of its use, either alone or in combination, in the treatment of leukemias and a range of solid tumors normally refractory to drug therapy.[3-5] Acute myelosuppression and concern about congestive heart failure are dose-limiting factors related to the use of this drug. Thus, anthracycline analogues which may further extend the antitumor spectrum of the parent antibiotic and/or reduce toxicity continue to be a worthwhile goal. Furthermore, although the mechanism of adriamycin-induced cardiomyopathy is not known, it has been suggested[6] that the development of cardiac toxicity may somehow be associated with the presence of the daunosamine moiety. Anthracycline analogues without the daunosamine moiety which continue to show biological activity may, if nothing else, contribute significantly to the elaboration of the molecular features which are associated with the problem of cardiotoxicity. The expectation that anthracycline analogues with fraudulent glycosides may be of value as antitumor agents derives, in practice, from

Scheme I



our own work with AD 32, a drug which does not bind to DNA, and from the retained antitumor activity of 7-*O*-β-D-glucosaminyldaunomycinone and 7-*O*-β-D-glucosaminyladriamycinone,[7] compounds which also do not fit the general accepted structure–activity hypothesis for adriamycin.[4] Furthermore, from theoretical considerations, it has been argued that conformational or configurational changes in the attached glycoside should continue to result

(1) A preliminary report of this work has appeared. See M. Israel and R. J. Murray in "Abstracts of Papers", 173rd National Meeting of the American Chemical Society, New Orleans, LA, Mar 1977, American Chemical Society, Washington, DC, 1977, Abstr MEDI 59.
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