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SYNTHESIS AND ANTITUMOR ACTIVITY OF 2′-BROMO- AND 2′-CHLORO-3′-ACETOXY-3′-DEAMINODAUNORUBICIN ANALOGS*

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ABSTRACT

Bromination of 3,4-di-*O*-acetyl-L-rhamnal (**7**) and subsequent glycosidic coupling under Koenigs–Knorr conditions with daunomycinone gave a mixture of three compounds having the β-L-*gluco* (**10**), α-L-*gluco* (**11**), and α-L-*manno* (**12**) configurations. Analogous bromination of 3,4-di-*O*-acetyl-L-fucal (**13**) followed by coupling with daunomycinone gave a mixture of three glycosides having the β-L-*galacto* (**16**), α-L-*galacto* (**17**), and α-L-*talo* (**18**) configurations. Chlorination of **7** and coupling with daunomycinone in the presence of silver triflate gave products having the α-L-*gluco* (**21**) and α-L-*manno* (**22**) configurations, whereas **13**, under similar conditions, gave only one stereoisomeric product, that having the α-L-*galacto* (**24**) configuration. Compounds **12** and **22** showed high *in vivo* activity in the P-388 lymphocytic leukemia assay.

INTRODUCTION

The recognized high activity of doxorubicin (**1**) and daunorubicin (**2**) against various types of human cancer, tempered by various undesirable side-effects[1], has motivated a search for analogs of greater efficacy and decreased cardiotoxicity. In this laboratory, systematic modification of these anthracycline glycosides in the carbohydrate portion has been conducted, together with some variations in the aglycon.

Previous publications[2,3] described the synthesis of the 3′-deamino-2′-iodo-analog (**5**) of daunorubicin (**2**) and of its 4′-epimer **6**. The biological properties of **5** and **6** established that a substituent at position 2′ does not inevitably lead to loss of antitumor activity. These analogs were synthesized because of the demonstrated activity of 3′-acetoxy[4] (**3**) and 3′-hydroxy[5] (**4**) doxorubicin analogs; the syntheses employed as precursors the glycal diacetates **7** and **13**. Biological tests showed that the introduction of an axial iodine atom at C-2′ *trans* to the glycosidic bond leads[3,6] to enhanced activity, especially in the L-rhamnal-derived compound **6**.

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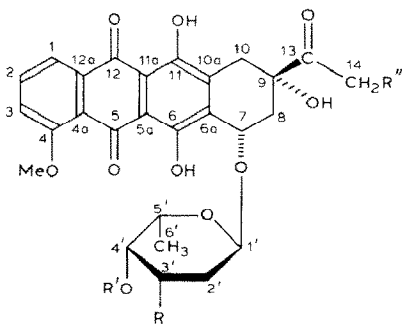

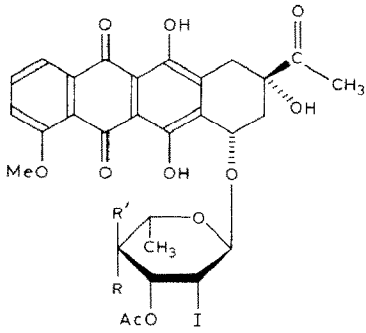

1 R = NH_2, R′ = H, R″ = OH
2 R = NH_2, R′ = H, R″ = H
3 R = OAc, R′ = Ac, R″ = OH
4 R = OH, R′ = H, R″ = OH

5 R = OAc, R′ = H
6 R = H, R′ = OAc

The present studies were undertaken to compare the biological effects as a function of the particular halogen at C-2′, and to determine how the configuration of the substituent at C-2′ influences activity. The syntheses performed led to the 2′-bromo and 2′-chloro analogs **10–12, 16–18, 21, 22**, and **24**. Comparative biological evaluation confirmed that the substituent at C-2′ must be axially oriented below the sugar ring for high activity. The 4′-epi compounds (derived from L-rhamnal) are significantly more active than those (derived from L-fucal) having the same spatial arrangement at C-4′ as the parent drugs; this observation parallels the behavior of the 4′-epimers of **1** and **2** with respect to the parent drugs themselves.

RESULTS AND DISCUSSION

Chemical synthesis. — The previously described[3] 2′-iodo derivatives **5** and **6** were prepared by oxyhalogenation of the appropriate glycal diacetates with *N*-iodosuccinimide in the presence of daunomycinone. *N*-Bromosuccinimide was successfully used in synthesis of certain 2′-bromoglycosides[7], but it failed to effect satisfactory coupling of the glycals **7** and **13** with daunomycinone.

A suitable alternative route used here employs direct bromination or chlorination of the 6-deoxyglycals diacetates **7** or **13** with molecular bromine or chlorine in the first step of a two-step sequence. Mixtures of stereoisomeric dihalogen adducts were formed whose proportions are dependent on the nature of the solvent and the configuration of the glycal[8].

Bromination of 3,4-di-*O*-acetyl-L-rhamnal (**7**) with bromine in carbon tetrachloride gave a mixture of two major adducts (**8** and **9**) in the ratio of ~2:1. These were brought into reaction with daunomycinone under Koenigs–Knorr conditions (HgO, $HgBr_2$) to give three diastereoisomers **10, 11**, and **12** in ~2:2:1 ratio. It is noteworthy that coupling of the α-L-*gluco* bromide **8** led to a 1:1 mixture of α and β anomers, whereas reaction with the α-L-*manno* dibromide **9** was more selective and only the α anomer (**12**) was isolated from the mixture.

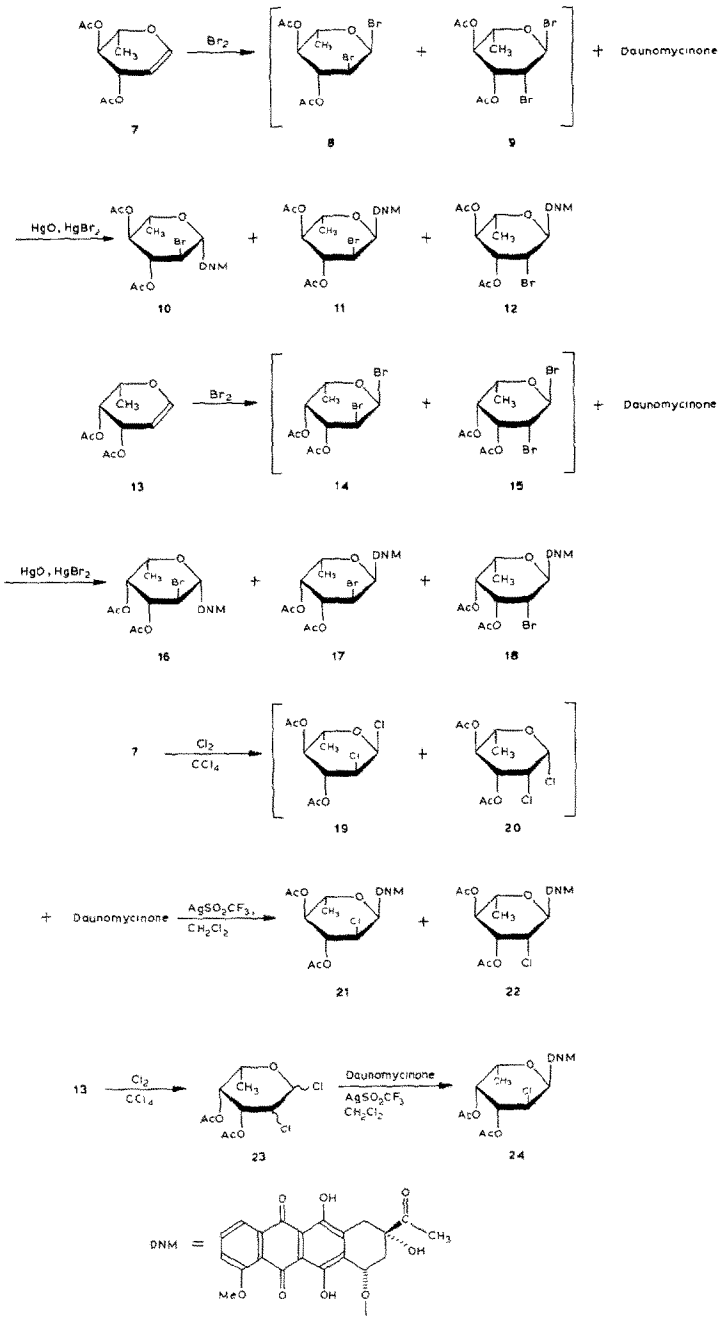
Br2
Daunomycinone
HgO, HgBr2
Cl2
CCl4
AgSO2CF3
CH2Cl2
DNM
MeO

TABLE I

^{1}H-NMR DATA [a] FOR COMPOUNDS **10**, **11**, **12**, **16**, **17**, **18**, **21**, **22**, AND **24**

Compound	*H-1* ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-1'* ($J_{1',2'}$)	*H-7*	*H-4'* ($J_{4',5'}$)	*H-3'* ($J_{3',4'}$)	*9-OH*	*H-5'* ($J_{5',6'}$)	*H-2'* ($J_{2',3'}$)	*H-10e* ($J_{8e,10e}$)	*H-10ax* ($J_{10ax,10e}$)	*H-8e* ($J_{8e,8ax}$)	*H-8ax* ($J_{8ax,8}$)	*H-6'*	*H-14*	*6-OH,11-OH*	*OMe*	*OAc*
10	8.06	7 77	7 39	5 08	5 56	4 66	5 28	4 42	3 60	3 76	3 24	3 01	2 57	2 39	1 04	2 42	14 10, 13 22	4 09	2 07
	(7 7)	(7 7)		(8 8)		(9 5)	(9 2)		(6 2)	(10 4)	(<1 5)	(19 5)	(14.7)	(<1.5 Hz)					2 01
11	8 04	7 78	7 39	5 57	5.50	4 77	5 26	4 28	4 21	3 92	3 30	3.05	— 2 13–2.31 —		1 26	2 45	14 06, 13 27	4 08	2 08
	(7 7)	(7 6)	(1 1)	(3 7)		(9 6)	(9 3)		(6 2)	(11 3)	(<1 5)	(19 1)							2 02
12	7 98	7 76	7 36	5 64	5 24	5 22	4 98	3 99	4 10	4 50	3 21	2 86	— 2 13–2.38 —		1 28	2 42	13 96, 13 18	4 07	2 06
	(7 7)	(8 0)	(1 1)	(1 5)		(9 6)	(9 6)		(6 2)	(4 0)	(<1 5)	(18 8)							2 03
16	8 04	7 79	7 40	5 08	5 57	5 11	5 13	4 55	3 45	3 75	3 27	3 06	— 2 70–2.40 —		1 00	2 44	14 14, 13 29	4 10	2 10
	(7 7)	(8 1)	(1 0)	(8 7)		(1 0)	(3.5)		(6 4)	(10 8)	(1 0)	(19 5)							2 05
17	8 03	7 78	7 39	5 65	5 50	5 30	5 14	4 28	4 43	4 21	3 28	3 02	— 2 00–2 35 —		1 24	2 43	14 05, 13.25	4 09	2 17
	(7 7)	(8 0)	(1 0)	(3 7)		(1 0)	(3 2)		(6 5)	(11 7)	(<1 0)	(19 2)							2 00
18	7 97	7 76	7 38	5 77	—	5 22 —	5 15	3 93	4 42	4 27	3 17	2 84	— 2 02–2 41 —		1 28	2 41	13 95, 13 14	4 08	2 02
	(7 7)	(8 1)	(0 9)	(~1 0)		(1 1)	(~3 0)		(6 5)	(~4 0)	(1 3)	(18 9)							2 02
21	8 00	7 76	7 38	5 55	5 48	4 78	5 20	4 08	4.19	3 90	3 26	3 00	— 2 05–2 32 —		1 26	2 44	14 01, 13 21	4 08	2 06
	(7 7)	(8 1)	(1 0)	(3 9)		(9 8)	(9 4)		(6 2)	(10 8)	(0 6)	(19 1)							2 02
22	7 98	7 76	7 38	5 54	5 24	5 21	5 13	3 97	4 09	4 45	3 20	2 84	— 2 06–2 32 —		1 28	2 43	13 96, 13 16	4 07	2 06
	(7 7)	(8 1)	(1 0)	(1 4)		(9 8)	(9 5)		(6 3)	(3 3)	(1 3)	(18 9)							2 03
24	8 02	7 77	7 39	5 61	5 48	5 33	5 09	4 09	4 42	4 18	3 26	3 01	— 2 00–2 29 —		1 23	2 43	14 02, 13 24	4 09	2 17
	(7 7)	(8 1)	(1 0)	(3 9)		(1 0)	(3 2)		(6 5)	(11 4)	(0 8)	(19 1)							2 00

[a] δ Values, coupling constants (in Hz) in parentheses

The β-L-*gluco* configuration of compound **10** was confirmed by ^{1}H-n.m.r. spectroscopy (Table I). The observed coupling constants $J_{1',2'}$ 8.8, $J_{2',3'}$ 10.4, $J_{3',4'}$ 9.2, and $J_{4',5'}$ 9.5 Hz indicated the axial disposition of H-1′, 2′,3′,4′, and 5′ in the $^1C_4$(L) conformation. Compound **10** also had a specific rotation ($[\alpha]_D$ +308°) identical to that of the 2′-iodo analog[3] (NSC 353 457) having the β-L-*gluco* configuration. Its α anomer **11** showed similar magnitudes of $J_{2',3'}$, $J_{3',4'}$, and $J_{4',5'}$ (11.3, 9.3, and 9.6 Hz, respectively), and the low $J_{1',2'}$ value of 3.7 Hz indicated the equatorial orientation of H-1′. Compound **12** displayed $J_{3',4'}$ and $J_{4',5'}$ = 9.6 Hz, establishing that H-3′, 4′, and 5′ are axially oriented in the $^1C_4$(L) conformation. The α-*manno* configuration was confirmed from the values of $J_{1',2'}$ (1.5) and $J_{2',3'}$ (4.0 Hz), which are in agreement with the values ($J_{1',2'}$ 1.3 and $J_{2',3'}$ 4.3 Hz) found for the 2-iodo-L-*manno* analog[3] (NSC 331 962). ^{13}C-N.m.r. data fully supported the assigned structures (Table II).

Compound **12** displayed high biological activity, and so further attempts were made to secure dibromide **9**, which was the desired precursor for a coupling reaction that would maximize the conversion of the expensive aglycon and save time-consuming and yield-decreasing separations. The use of nitromethane instead of carbon tetrachloride as solvent for the bromine addition changed[8] the ratio of dibromides **8** and **9** from ~2:1 to ~1:1. Subsequent rapid chromatography gave a mixture enriched in the α-L-*manno* dibromide **9**. Coupling of this material with daunomycinone under the conditions already described gave compound **12** in 48% yield.

Synthesis of the 2′-bromo analogs having the L-*galacto* and L-*talo* configurations started from 3,4-di-*O*-acetyl-L-fucal (**13**), which was brominated in carbon tetrachloride at 0°. Two isomeric dibromides, the α-L-*galacto* (**14**) and α-L-*talo* (**15**) adducts, were the principal products, obtained in the ratio of ~3:2. Direct coupling of this mixture with daunomycinone in dichloromethane, in the presence of yellow mercuric oxide and mercuric bromide in dichloromethane, as in the reaction starting from glycal **7**, gave three diastereoisomeric glycosides **16, 17**, and **18** in ~2:6:3 ratio. The difference in configuration at C-4 seems to be responsible for the observed increase in the proportion of α anomer **17**.

Compound **16** has H-2′ and H-3′ axially oriented ($J_{2',3'}$ 10.8 Hz) in the expected $^1C_4$(L) conformation, and thus the L-*galacto* configuration may be assigned. The large $J_{1',2'}$ coupling (8.7 Hz) indicates that compound **16** is the β anomer. The large value of $J_{2',3'}$ (11.7 Hz) and the smaller magnitude of $J_{1',2'}$ (3.7 Hz) indicates the α-L-*galacto* configuration for compound **17**. The structure of compound **18** was assigned as α-L-*talo* on the basis of the small values of $J_{1',2'}$ (1.0 Hz) and $J_{2',3'}$ (4.0 Hz), and by comparison of other data for **18** with those[3] for compound **5**.

Chlorination of 3,4-di-*O*-acetyl-L-rhamnal (**7**) gave a higher proportion of the L-*gluco* isomer (**19**) than was observed in comparable bromination. On the basis of ^{1}H-n.m.r. spectral integrals, the ratio between the L-*gluco* (**19**) and L-*manno* (**20**) dichlorides was 4:1. This mixture was coupled with daunomycinone in the presence

TABLE II

^{13}C-N.M.R. DATA (δ) FOR COMPOUNDS **10**, **11**, **12**, **16**, **17**, **18**, **21**, **22**, AND **24**[a]

C Atom	*Compound*								
	10	**11**	**12**	**16**	**17**	**18**	**21**	**22**	**24**
1	120.0	120.0	119.0	120.0	120.0	119.9	119.9	119.9	119.8
2	135.8	135.8	135.8	135.0	135.8	135.8	135.8	135.8	135.7
3	118.7	118.7	118.6	118.7	118.7	118.7	118.6	118.6	118.5
4	161.4	161.4	161.3	161.4	161.4	161.4	161.3	161.3	161.2
6	156.8	156.2	156.3	156.9	156.3	156.4	156.3	156.3	156.2
11	155.9	155.8	155.7	156.0	155.8	155.8	155.8	155.7	155.7
5	187.3	187.4	187.2	187.4	187.3	187.2	187.3	187.2	187.2
12	187.0	187.2	187.0	187.0	187.1	187.0	187.1	187.0	187.0
4a	121.2	121.3	121.2	[b]	[b]	121.1	121.2	121.0	121.1
5a	111.7	111.9	111.9	111.7	111.9	111.8	111.9	111.7	111.7
11a	111.3	111.7	111.8	111.3	111.7	111.7	111.7	111.6	111.5
6a	136.1	135.8	135.8	136.1	135.8	135.7	135.8	135.6	135.7
10a	135.8	135.4	134.4	135.0	135.3	134.4	135.3	135.4	135.1
12a	133.3	133.1	133.2	133.6	133.3	133.3	133.1	133.1	135.1
7	70.1	68.3	71.2	70.4	68.2	71.2[c]	68.6	71.1[c]	68.3
8	34.0[c]	35.5	35.4	34.2	35.4	35.1	35.4	35.1	35.3
9	77.2	76.5	[d]	[d]	76.6	76.4	75.6	[d]	76.4
10	33.9[c]	34.0	33.4	34.0	34.0	33.1	33.9	34.4	33.8
13	212.6	212.0	211.3	212.6	212.0	211.6	212.0	211.6	211.9
14	24.7	24.6	24.6	24.7	24.6	24.4	24.6	24.4	24.5
OMe	56.7	56.7	56.8	56.7	56.7	56.6	56.6[c]	56.5	56.5
1′	102.4	98.5	103.3	103.3	99.3	104.6	98.7	103.1	99.3
2′	50.7	47.3	49.8	49.6	45.6	44.3	56.6[c]	56.5	56.5
3′	74.9	71.8	69.2	73.7[c]	70.0	66.0[c]	71.8	69.4[c]	69.9
4′	74.2	74.5	71.2	70.6[c]	71.4	68.3[c]	74.3	70.4[c]	71.0
5′	70.5	66.6	68.3	69.9	65.7	65.9[c]	66.4	68.0[c]	65.5
Me-5′	17.1	17.0	17.5	15.7	15.8	15.9	17.0	17.2	15.6
OAc		20.5				20.6	20.5	20.5	
	20.4		20.7	20.3	20.4				20.2
OAc		20.4				20.4	20.4	20.4	
C=O	170.0	170.0	169.7	170.6	170.5	170.7	170.0	169.9	170.4
C=O	169.9	169.7		169.8	169.7	169.7	169.9	169.8	169.7

[a]All spectra were recorded for solutions in (^{2}H)chloroform. Chemical-shift assignments are based on off-resonance decoupling plus single-frequency, selective heteronuclear decoupling and comparison with literature values[3]. Assignments bracketed are not specifically differentiated. [b]Low in intensity. [c]Assignments may be interchanged. [d]Overlapped with $CDCl_3$ signals.

of silver triflate; use of mercuric salts in this instance gave very low yields. As expected, the change of coupling reagents affected the stereoselectivity of the reaction; the only products isolated were the α anomers **21** and **22**, formed in 3:2 ratio. The ^{1}H-n.m.r. data for compound **21** were very close to those of the 2′-bromo analog **11**, and epimer **22** likewise had coupling constants almost identical to those of its 2′-bromo counterpart **12**. Furthermore, the ^{13}C-n.m.r. chemical shifts of C-1′ for **11** and **21**, and **12** and **22**, were closely related (Table II). These considerations

led to the assignment of the α-L-*gluco* configuration to compound **21** and α-L-*manno* for compound **22**.

The marked biological activity of 7-*O*-(3,4-di-*O*-acetyl-2-chloro-2,6-dideoxy-α-L-mannopyranosyl)daunomycinone (**22**, NSC 354 320) prompted the development of an improved synthesis. The mixture of dichlorides **19** and **20** was chromatographed to give the crystalline isomer **20**, which reacted readily with daunomycinone in the presence of silver triflate to give compound **22** in 66% yield.

Chlorination of di-*O*-acetyl-L-fucal (**13**) gave a mixture of adducts (**23**) in which the L-*galacto* isomers were preponderant[8] (~85%). This mixture of dihalides was then coupled with daunomycinone in the presence of silver triflate to afford 7-*O*-(3,4-di-*O*-acetyl-2-chloro-2,5-dideoxy-α-L-galactopyranosyl)daunomycinone (**24**) in 44% yield. The assigned structure was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy, and by comparison with the configurationally related compound **17** (Tables I and II).

Biological activity. — All compounds in this study having the L-*gluco* and L-*galacto* configurations (**10, 11, 16, 17, 21**, and **24**), and thus having a bromine or chlorine atom equatorially oriented at C-2′, regardless of anomeric configuration, were inactive and non-toxic at doses up to 50 mg/kg in the *in vivo* murine P-388 lymphocytic leukemia test*. Their 2′-epimers (axial halogen at C-2′) were all active. The activity was moderate for the 2′-bromo-α-L-*talo* analog **18** (NSC 353 450, T/C 145 at 50 mg/kg, the highest dose tested)†, and very high for the 4′-epimers **12** and **22** (having the α-L-*manno* configuration). The 2′-bromo analog **12** (NSC 354 461) showed T/C 245 at 25 mg/kg† with the first injection on day 5, and T/C 278 at 25 mg/kg and 229 at 12.5 mg/kg with injections on days 1, 5, and 9. Compound **22** (NSC 354 320), which has a chlorine atom at C-2′, showed T/C 248 at 25 mg/kg. No significant differences in the P-388 test-results were observed between compounds **12** and **22**, and their 2′-iodo counterpart[3] **6**, and thus the nature of the halogen atom appears to have little influence on activity. These data and data previously reported for the 2′-iodo congeners[3,6] showed that these 2′-halo anthracycline analogs are most active when the sugar has the α-L-*manno* configuration, are moderately active when the configuration is α-L-*talo,* and are completely devoid of activity at doses up to 50 mg/kg for comparable structures having the L-*gluco* and L-*galacto* configurations.

EXPERIMENTAL

General methods. — T.l.c. was performed on precoated plates of Silica gel 60 (E. Merck, Darmstadt, Germany); zones of colorless compounds were detected by

*Screened by Adria Laboratories, Inc., Dublin, Ohio. Test conditions: One intraperitoneal injection on day 5.

†Data obtained under the auspices of the U.S. National Cancer Institute, Division of Cancer Treatment, Drug Research and Development Branch. Test conditions: 3 injections (intraperitoneal) on days 5, 9, and 13.

spraying the plates with H_2SO_4 and subsequent heating. Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. and ^{13}C-n.m.r. spectra at 200 and 50 MHz, respectively, were recorded by Drs. O. Mols and P. Bhaté with a Bruker WP-200 spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane (δ 0.00). Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, Georgia.

7-O-(3,4-Di-O-acetyl-2-bromo-2,6-dideoxy-β-L-glucopyranosyl)daunomycinone (**10**), *7-O-(3,4-di-O-acetyl-2-bromo-2,6-dideoxy-α-L-glucopyranosyl)daunomycinone* (**11**), *and 7-O-(3,4-di-O-acetyl-2-bromo-2,6-dideoxy-α-L-mannopyranosyl)daunomycinone* (**12**, *NSC 354 461*). — A solution of 3,4-di-*O*-acetyl-L-rhamnal (**7**; 0.6 g, 2.8 mmol) in carbon tetrachloride (14 mL), protected from light, was cooled in an ice–water bath, and Br_2 was added dropwise until a slight red color appeared. The solution was kept for 10 min at 0° and evaporated under diminished pressure, affording a product whose ^{1}H-n.m.r. spectrum showed it to be a mixture of 1,2-dibromides having the α-L-*gluco* (**8**) and α-L-*manno* (**9**) configurations in ~2:1 ratio. The syrup was dissolved in dichloromethane (30 mL) and the solution was added to a vigorously stirred suspension of daunomycinone (0.7 g, 1.75 mmol), yellow HgO (1.4 g, 6.45 mmol), $HgBr_2$ (0.36 g, 1 mmol), and 4A molecular sieves (12 g) in dichloromethane (120 mL). After stirring for 20 h, t.l.c. examination of the mixture showed traces of daunomycinone and three faster-moving red spots (R_F 0.55, 0.50, and 0.44 in 3:1 toluene–acetone). The mixture was filtered through Celite, and the residue washed with dichloromethane until the filtrate became colorless. The combined filtrate (~300 mL) was washed with 10% aqueous KI (100 mL, twice) and water (100 mL), and then dried ($MgSO_4$), and evaporated.

The mixture was resolved by column chrommography on silica gel (100 g) with 6:1 toluene–acetone as eluant. The slowest-moving component (daunomycinone, R_F 0.23) was isolated as a red powder (0.12 g). The yields of the other products are based on the daunomycinone that reacted (0.58 g, 1.46 mmol).

The first (R_F 0.55) and third (R_F 0.44) fractions were pure (t.l.c.), but the intermediate fraction (R_F 0.50) was contaminated by the two others, and so it was rechromatographed on a column of silica gel (50 g) that was eluted with 6:1 toluene–acetone. The pure fractions from both columns were collected and evaporated.

The first fraction afforded the β-L-*gluco* derivative **10** as a syrup, which was dissolved in a small amount of chloroform. The product was precipitated by ether–hexane; yield 267 mg (27%), m.p. 235° (dec.), $[\alpha]_D^{25}$ +308° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13}$ (691.482): C, 53.85; H, 4.52; Br, 11.56. Found: C, 53.63; H, 4.55; Br, 11.63.

Evaporation of the second fraction afforded the α-L-*gluco* derivative **11**, which precipitated from a concentrated solution in chloroform by addition of hexane; yield 286 mg (28%), m.p. 135–137° (dec.), $[\alpha]_D^{25}$ +269° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13} \cdot 0.5\,H_2O$ (700.48): C, 53.15; H, 4.61; Br, 11.41. Found: C, 53.04; H, 4.63; Br, 11.41.

The α-L-*manno* derivative (**12**) was isolated from the third fraction from the column by precipitation from chloroform solution with hexane; yield 132 mg (13%), m.p. 146–147°, $[\alpha]_D^{25}$ +173° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13}$ (691.482): C, 53.85; H, 4.52; Br, 11.56. Found: C, 53.76; H, 4.54; Br, 11.64.

*Direct preparation of 7-O-(3,4-di-O-acetyl-2-bromo-2,6-dideoxy-α-L-manno-pyranosyl)daunomycinone (**12**, NSC 354 461).* — A solution of L-rhamnal diacetate (**7**; 5.35 g, 25 mmol) in nitromethane (125 mL) was treated as before with Br_2 for 10 min at 0°. The syrup obtained after evaporation of the solvent consisted of a ~1:1 mixture of α-L-*gluco* (**8**) and α-L-*manno* (**9**) dibromides. Purification by column chromatography on silica gel (150 g) with 5:1 hexane–ethyl acetate as eluant afforded a fraction enriched in the α-L-*manno* dibromide (**9**:**8**, ~3:1). Evaporation gave 3.3 g of a syrup, 3.0 g (8 mmol) of which was dissolved in dichloromethane (50 mL) and was added to a magnetically stirred suspension of yellow HgO (4.2 g), $HgBr_2$ (1.1 g), daunomycinone (2.0 g, 5 mmol), and 4A molecular sieves (15 g) in dichloromethane (400 mL). After 20 h, the mixture was processed as in the previous experiment. Resolution by column chromatography (silica gel, 300 g; 6:1 toluene–acetone) yielded 0.4 g of unreacted daunomycinone and compound **12** (1.34 g, 48% based on reacted daunomycinone) as a red powder.

7-O-(3,4-Di-O-acetyl-2-bromo-2,6-dideoxy-β-L-galactopyranosyl)daunomycinone (**16**), *7-O-(3,4-di-O-acetyl-2-bromo-2,6-dideoxy-α-L-galactopyranosyl)-daunomycinone* (**17**), *and 7-O-(3,4-di-O-acetyl-2-bromo-2,6-dideoxy-α-L-talo-pyranosyl)daunomycinone (**18**, NSC 353 450).* — Bromination of 3,4-di-*O*-acetyl-L-fucal (**13**; 0.6 g, 2.8 mmol) in carbon tetrachloride, for 10 min at 0° with exclusion of light, afforded a mixture of α-L-*galacto* and α-L-*talo* dibromides (~3:2 by ^1H-n.m.r.). This syrupy mixture, dissolved in dichloromethane (30 mL), was added to a magnetically stirred suspension of yellow HgO (1.4 g, 6.45 mmol), $HgBr_2$ (0.36 g, 1 mmol), 4A molecular sieves (12 g), and daunomycinone (0.7 g, 1.75 mmol). The mixture was stirred for 20 h at room temperature, whereupon t.l.c. showed traces of unreacted daunomycinone and three faster-moving components having R_F 0.60, 0.57, and 0.52. The suspension was filtered through Celite and the residue washed with dichloromethane. The filtrate (~250 mL) was washed with 10% KI (100 mL, twice), water (100 mL), dried ($MgSO_4$), and evaporated. The mixture was resolved by column chromatography (silica gel, 120 g) with 8:1 toluene–acetone as eluant. The pure fractions were collected and those that contained two or more components were combined, evaporated, and rechromatographed (silica gel, 50 g; 6:1 toluene–acetone).

Fractions containing the fastest-moving component (R_F 0.60) were combined and evaporated. The residue was dissolved in a small amount of chloroform and compound **16** precipitated with ether–hexane; yield 170 mg (14%), m.p. 142–144°, $[\alpha]_D^{25}$ +288° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13}$ (691.482): C, 53.85; H, 4.52; Br, 11.56. Found: C, 53.55; H, 4.57; Br, 11.63.

The product in the second fraction (R_F 0.57) was precipitated from chloroform–ether by adding hexane, affording compound **17** (0.5 g, 41%), m.p. 153–155°, $[\alpha]_D^{25}$ +264° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13}$ (691.482): C, 53.85; H, 4.52; Br, 11.56. Found: C, 53.66; H, 4.55; Br, 11.62.

The slowest-moving fraction (R_F 0.52) afforded, after evaporation, dissolution in chloroform, and addition of hexane, compound **18** as a red powder (250 mg, 21%); m.p. 154–157°, $[\alpha]_D^{25}$ +162° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}BrO_{13}$ (691.482): C, 53.85; H, 4.52; Br, 11.56. Found: C, 53.66; H, 4.56; Br, 11.52.

7-O-(3,4-Di-O-acetyl-2-chloro-2,6-dideoxy-α-L-glucopyranosyl)daunomycinone (**21**) *and 7-O-(3,4-di-O-acetyl-2-chloro-2,6-dideoxy-α-L-mannopyranosyl)daunomycinone* (**22**, *NSC 354 320*). — 3,4-Di-*O*-acetyl-L-rhamnal ((**7**; 0.86 g, 4 mmol) was dissolved in carbon tetrachloride (20 mL) and Cl_2 was bubbled through the solution at 0° in the dark. After 10 min at 0°, the solvent was evaporated and the residue crystallized spontaneously. It was suspended in pentane and filtered; yield 1.0 g (88%) of colorless crystals. ^{1}H-N.m.r. showed a mixture of α-L-*gluco* **19** and α-L-*manno* **20** in 4:1 ratio.

The mixed dihalides **19** and **20** (0.6 g, 2.1 mmol) and daunomycinone (0.7 g, 1.75 mmol) were dissolved in dichloromethane (150 mL). The solution was vigorously stirred and silver triflate (0.46 g, 1.8 mmol) dissolved in oxolane (2.4 mL) was added in three portions (every 10 min). After 0.5 h, almost 50% of the daunomycinone had reacted, and the mixture was filtered through Celite. Conducting the reaction for longer periods did not raise the yield of glycosides, but by-products appeared. The filtrate was washed with saturated aqueous $KHCO_3$ (50 mL, twice), water (100 mL), dried ($MgSO_4$), and evaporated.

Chromatography of the mixture (silica gel, 150 g; 8:1 toluene–acetone) gave two main products and unreacted daunomycinone (0.32 g). The yields of the products are based on the amount of daunomycinone that had reacted.

The first fraction afforded compound **21**, which precipitated from chloroform with ether–hexane; yield 0.14 g (23%), m.p. 135–137°, $[\alpha]_D^{25}$ +240° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}ClO_{13}$ (647.031): C, 57.55; H, 4.83; Cl, 5.48. Found: C, 57.47; H, 4.85; Cl, 5.54.

Evaporation of the second fraction and precipitation of the product from chloroform by adding ether–hexane afforded 0.10 g (16%) of compound **22**; m.p. 144–146°, $[\alpha]_D^{25}$ +229° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}ClO_{13}$ (647.031): C, 57.55; H, 4.83; Cl, 5.48. Found: C, 57.38; H, 4.85; Cl, 5.57.

Direct preparation of 7-O-(3,4-di-O-acetyl-2-chloro-2,6-dideoxy-α-L-mannopyranosyl)daunomycinone (**22**, *NSC 354 320*). — L-Rhamnal diacetate (**7**; 3.0 g, 14

mmol) was chlorinated as already described. The crystalline mixture of dichlorides was separated by column chromatography (silica gel, 180 g; 6:1 hexane–acetone). Compound **20**, having the L-*manno* configuration, was obtained crystalline in ~10% yield. The dichloride **20** (0.35 g, 1.1 mmol) was poured into a solution of daunomycinone (0.3 g, 0.75 mmol) in dichloromethane (60 mL). The solution was vigorously stirred and silver triflate (260 mg, 1 mmol), dissolved in oxolane (2.4 mL), was added in 0.8-mL portions every 10 min. After 30 min, AgCl that had precipitated was filtered off, and the filtrate was treated as in the previous preparation. Compound **22** was purified by column chromatography, the fractions were evaporated, and the residue was dissolved in a small amount of chloroform and precipitated with ether–hexane. From the column, 60 mg of unreacted daunomycinone was recovered. Compound **22** was obtained in 66% yield (0.28 g).

7-O-(3,4-Di-O-acetyl-2-chloro-2,6-dideoxy-α-L-galactopyranosyl)daunomycinone (**24**). — 3,4-Di-*O*-acetyl-L-fucal (**13**; 0.54 g, 2.5 mmol) dissolved in carbon tetrachloride (15 mL), was treated with Cl_2 for 10 min at 0° in the dark. The solution was evaporated and the colorless, syrupy dichlorides **23**, dissolved in dichloromethane (50 mL), were added to a solution of daunomycinone in dichloromethane (100 mL). The mixture was vigorously stirred and silver triflate (0.46 g, 1.8 mmol), dissolved in oxolane (2.4 mL), was added in 0.8-mL portions every 10 min. After 0.5 h, the precipitate of AgCl was filtered off and the filtrate was washed with saturated aqueous $KHCO_3$ (50 mL, twice), water (100 mL), dried ($MgSO_4$), and evaporated. Purification by column chromatography (silica gel, 150 g; 8:1 toluene–acetone) afforded one major component and unreacted daunomycinone (0.18 g). Compound **24** precipitated from a concentrated solution in chloroform by addition of ether–hexane; yield 0.4 g (44%), m.p. 146–148°, $[\alpha]_D^{25}$ +246° (*c* 0.02, chloroform).

Anal. Calc. for $C_{31}H_{31}ClO_{13}$ (647.031): C, 57.55; H, 4.83; Cl, 5.48. Found: C, 57.26; H, 4.86; Cl, 5.55.

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