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Synthesis and antitumor activity of new D-galactose-containing derivatives of doxorubicin

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Abstract

A general scheme of synthesis of antibiotic doxorubicin derivatives is based on the 13-dimethyl ketal of 14-bromodaunorubicin (**4**). The interaction of **4** with melibiose (**5**), lactose (**6**), 3-methoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde (**12**) or 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde (**13**) by reductive alkylation followed by hydrolysis of the corresponding intermediate bromoketals produced 3′-*N*-[α-D-galactopyranosyl-(1→6)-*O*-1-deoxy-D-glucit-1-yl]doxorubicin (**7**), 3′-*N*-[β-D-galactopyranosyl-(1→4)-*O*-1-deoxy-D-glucit-1-yl]doxorubicin (**8**), 3′-*N*-[3″-methoxy-4″-*O*-(β-D-galactopyranosyl)-4″-oxybenzyl]doxorubicin (**16**), and 3′-*N*-[4″-*O*-(β-D-galactopyranosyl)-4″-oxybenzyl]doxorubicin (**17**). Cytotoxic and antitumor activity of the synthesized drug candidates compared to the parent doxorubicin was studied using various experimental models, in particular, on mice bearing lymphocyte leukemia P-388 at single and multiple i.v. injection regimens.

Keywords: Doxorubicin; Reductive alkylation with mono- and disaccharides; D-Galactose; Lactose; Meliobiose

1. Introduction

Chemical modification of anthracycline antibiotics remains important for the development of compounds that overcome natural and induced resistance to the existing compounds. Many researchers in the field are currently focused on the synthesis and study of hydrophobic derivatives of anthracycline antibiotics, some of which seem to be poor substrates of MDR-determining glycoproteins.[1] However, the introduction of an additional sugar residue may also lead to valuable compounds as a sugar may enhance the antitumor activity of a modified antibiotic through interaction with specific receptors in tumor cells.[2–4] In recent years, a series of anthracycline derivatives containing sugar moieties connected to the antibiotic through alkyl- or acyl-type spacers were synthesized in connection with gene-directed enzyme prodrug therapy (GDEPT).[5,6] In these cases, the sugar moieties serve as enzyme-specific functional groups of the anthracycline substrate. When a spacer is hydrolyzed by specific enzymes in the target tissue, the inactive prodrug is activated into the initial drug. The goal of our research, in contrast, was to modify the initial drug with a stable galactose residue, aiming at reducing the drug toxicity, increasing its efficacy, or both. The galactose residue was chosen because of the important role that galactose-specific receptors, galectins, play in tumor development.[7,8]

Here, we report the synthesis of new N-substituted derivatives of doxorubicin containing D-galactose with hydrophilic (1-deoxyglucit-1-yl) or hydrophobic (benzyl or 3-methoxybenzyl) spacers, and test their cytotoxic and antitumor activity as compared to the parent doxorubicin. Up to now no methods for the conjugation of the 3-amino group of daunosamine with aldoses have been published.

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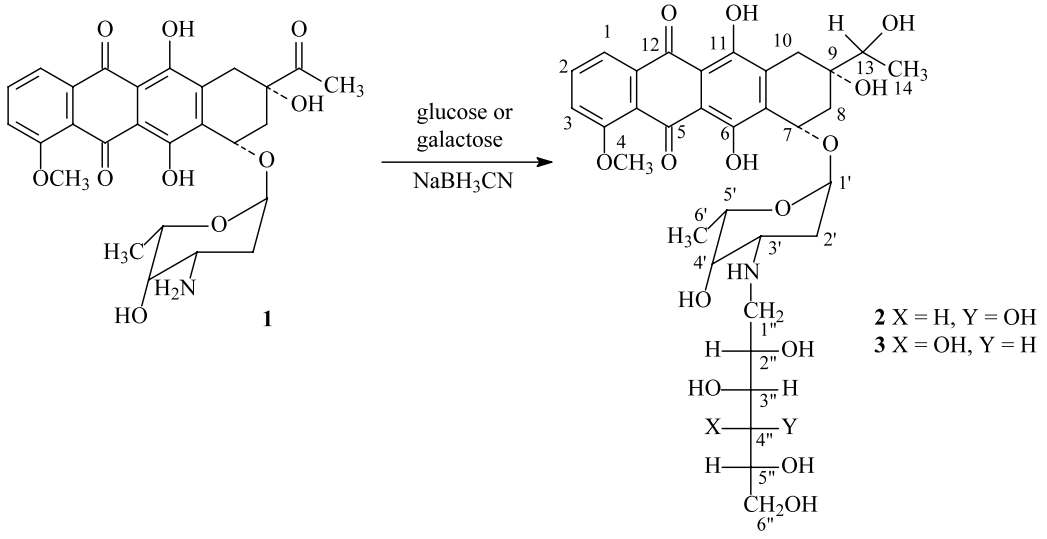
2. Results and discussion

In the first stage of our research, we studied the possibility of 3′-N-substitution of daunorubicin (**1**) by reductive alkylation of **1** using D-glucose or D-galactose and $NaBH_3CN$. Although the reductive N-alkylation of anthracycline antibiotics has been widely studied neither mono- nor disaccharides have been used in this reaction. By this method, 3′-(1-deoxy-D-glucit-1-yl)- and 3′-(1-deoxy-D-galactit-1-yl) derivatives of 13-dihydro-13-(*RS*)-daunorubicin (**2**, **3**) were isolated in ~20% yields (Scheme 1). To protect the 13-CO group of the antibiotic from the reduction, the 13-dimethyl ketal of 14-bromodaunorubicin (**4**) was used as the starting compound, obtained from daunorubicin (**1**) by described methods.[9,10] To introduce the D-galactose substituent, the disaccharides 6-*O*-α-D-galactopyranosyl-D-glucose (melibiose) (**5**) and 4-*O*-β-D-galactopyranosyl-D-glucose (lactose) (**6**) were used. A modified procedure, previously described for the reductive amination of oligosaccharides with proteins in the presence of $NaCNBH_3$, was used.[11] 3′*N*-[α-D-Galactopyranosyl-(1→6)-*O*-1-deoxy-D-glucit-1-yl]doxorubicin (**7**) and 3′-*N*-[β-D-galactopyranosyl-(1→4)-*O*-1-deoxy-D-glucit-1-yl]doxorubicin (**8**) were obtained in 20 and 8% yields, respectively starting from **4** and melibiose (**5**) or lactose (**6**) with the use of $NaBCNH_3$ after hydrolysis of the intermediate bromoketals **7a** and **8a** (Scheme 2). D-Galactose has the α-anomeric configuration in compound **7** and the β configuration in compound **8**; the polyhydroxylated hexit-1-yl spacer in compound **8** is shorter and more branched as compared to that in compound **7**.

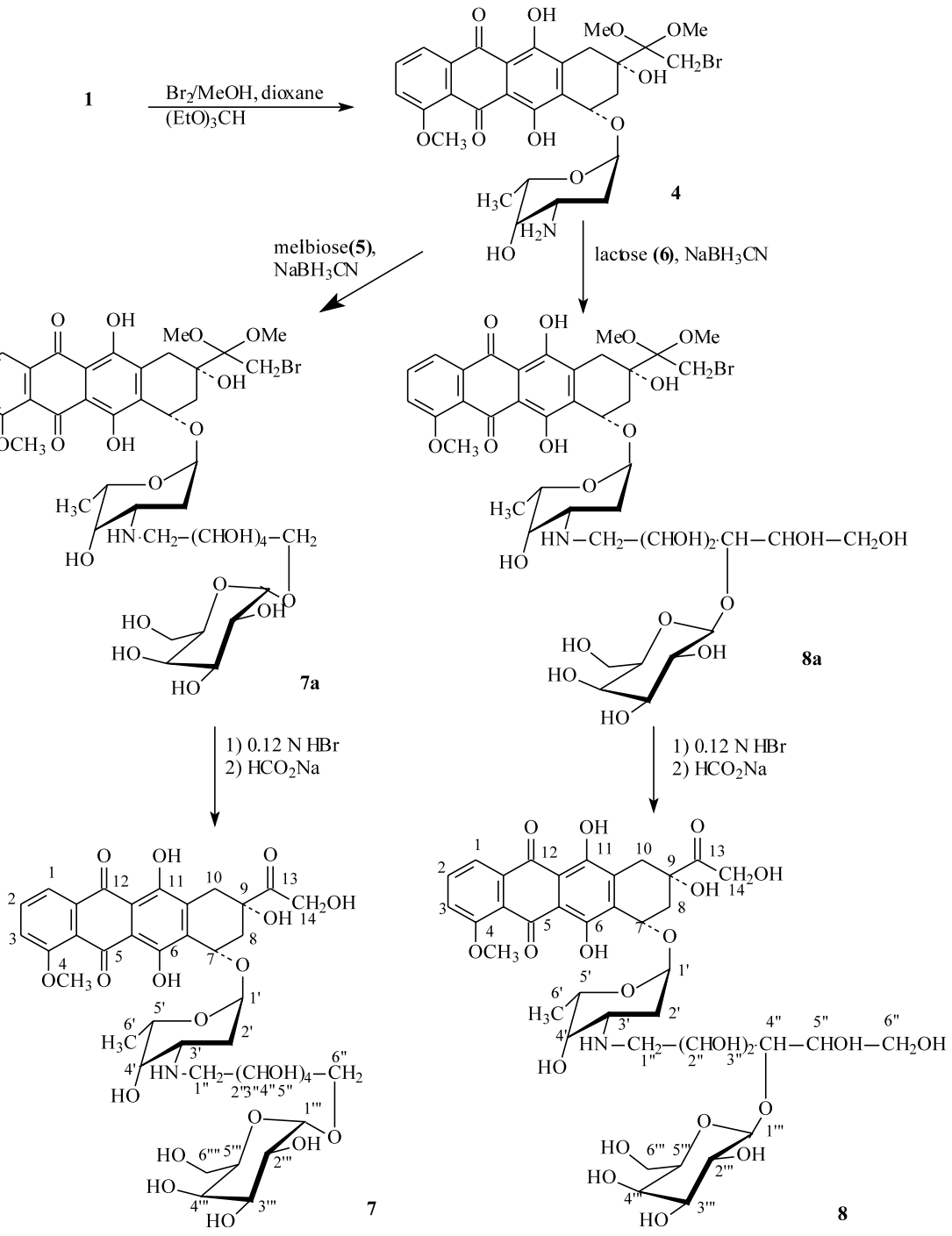
In the first stage of the synthesis of the conjugates **16** and **17** of doxorubicin with D-galactose linked to the antibiotic through the more hydrophobic spacer, 3-methoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxybenzaldehyde (**12**) and 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)oxybenzaldehyde (**13**) were obtained by the reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11**) with vanillin (**9**) or 4-hydroxybenzaldehyde (**10**), respectively (Scheme 3). Reductive alkylation of the 3′-amino group of **4** with compound **12** or **13** by the use of $NaBCNH_3$ gave the corresponding derivatives **14** and **15** of the 13-dimethylketal of 14-bromodaunorubicin. After deacetylation of the galactose moiety in **14** and **15** with NaOMe in methanol followed by acid hydrolysis of the intermediates **14a** and **15a**, the desired doxorubicin derivatives **16** and **17** were obtained (Scheme 3).

3′-*N*-(1-Deoxy-D-glucit-1-yl)doxorubicin (**18**) and 3′-*N*-(1-deoxy-D-galact-1-yl)doxorubicin (**19**) (Scheme 4) were obtained from **4** and D-glucose or D-galactose each in 5% yields.

Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses showed that compounds **7**, **8**, and **16** through **19** were homogeneous, and contain no admixed daunorubicin or doxorubicin. Under conditions of drastic acid hydrolysis (1 N HCl, 105 °C, 1 h) compounds **7**, **8**, **16**, and **17** produce the aglycon adriamycinone plus galactose, as demonstrated by TLC and paper chromatography, using authentic compounds as standards. Compounds **2** and **3** under similar conditions produced 13-(*R,S*)-dihydrodaunomycinone, and compounds **18** and **19** gave adriamycinone. NMR investigations permitted identification of all signals in the agycon, spacers, and carbohydrate moieties (Table 1), and mass-spectral data showed the correct molecular weights.



Scheme 1.

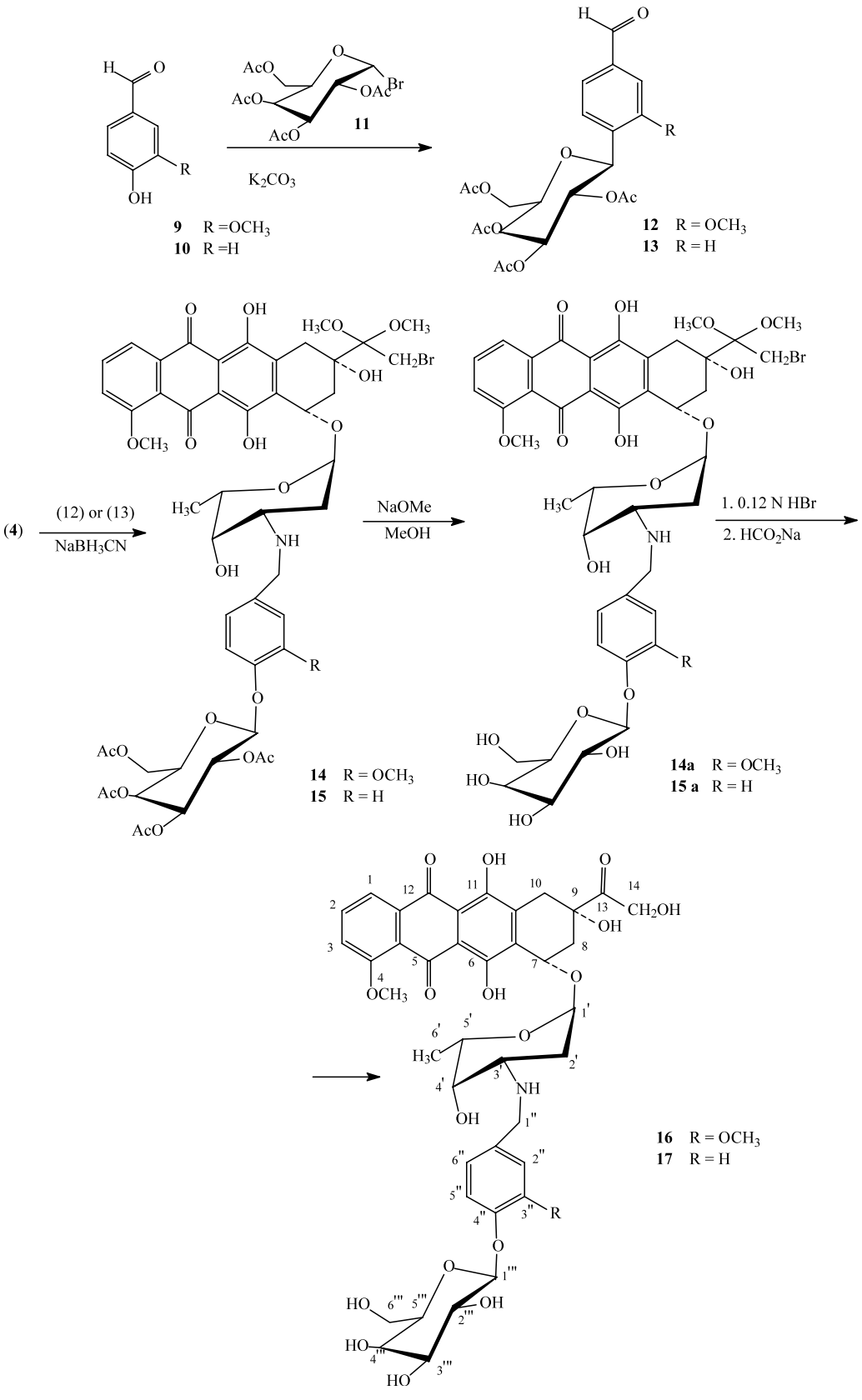


Scheme 2.

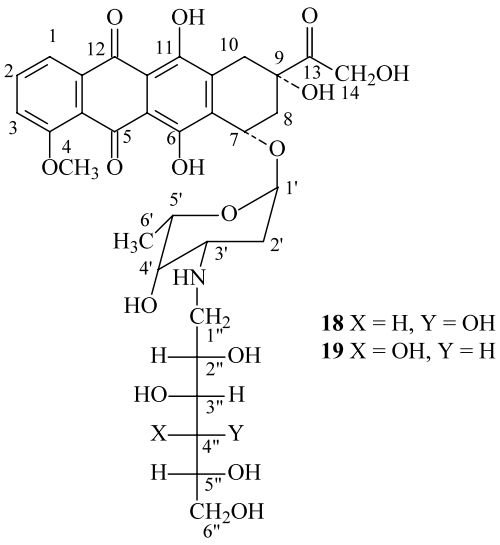
3. Antitumor activity

The in vitro inhibitory effects of **7** and **8** on the proliferation of murine leukemia L1210/0 showed that these compounds are two orders less cytotoxic than doxorubicin: for **7** IC_{50} (50% inhibitory concentration) = 21 μM (L1210/0) and for **8** $IC_{50} = 24$ μM, whereas for doxorubicin shows $IC_{50} = 0.213$ μM.

In vivo studies revealed differences in the antitumor properties of compounds **7** and **8**. The maximum tolerated dosages (MTD) were 40–60 mg/kg for **7**, 60–80 mg/kg for **8**, and >60 mg/kg for both **16** and **17**


11
K2CO3
9 R =OCH3
10 R =H
12 R = OCH3
13 R = H
(4)
(12) or (13)
NaBH3CN
NaOMe
MeOH
1. 0.12 N HBr
2. HCO2Na
14 R = OCH3
15 R = H
14a R = OCH3
15 a R = H
16 R = OCH3
17 R = H

Scheme 3.



Scheme 4.

(single i.v. injection, BDF_1 mice ($C_{57}Bl \times DBA_2$, males), as compared to 7–10 mg/kg for doxorubicin. The antitumor activity for these compounds was studied on mice bearing lymphocyte leukemia P-388 at single and multiple (q2d × 3) i.v. injection regimens (Table 2).

3.1. Single injection regimen

When **8** was i.v. injected to mice with P-388 (BDF_1 mice) 24 h post i.p. implantation of the tumor, 65% ILS at the dose of 40 mg/kg was achieved. Compound **7** was more active than **8**: at a dose of 20 mg/kg it induced 79% ILS and at 40 mg/kg 118% ILS (without toxic effects). A dose of 60 mg/kg showed some toxicity. The maximal antitumor effect of doxorubicin was 70% at the MTD dose of 7 mg/kg. Compounds **16** and **17** at doses of 40 and 60 mg/kg induced ILS in the range 35–44% (**16**) and 52–59% (**17**), respectively.

3.2. Multiple injection regimen

Multiple injections of doxorubicin revealed an increased toxicity of the drug, apparently due to its cumulative toxic effect. Compared to a single dose regimen, when at the dose of 7 mg/kg there was no deaths (70% ILS), triple injection of doxorubicin with 2-day intervals (q2d × 3) at 2.3 mg/kg each dose (6.9 mg/kg total dose) resulted in four toxic deaths out of seven animals. However, compound **7** did not show any cumulative toxic effect at the triple dose of 40 mg/kg (120 mg/kg total dose), and induced ILS equal to 133%. Hence, compound **7** may be of interest for supportive therapy.

This study shows that doxorubicin derivatives containing at the nitrogen atom of the daunosamine moiety a polyhydroxylated spacer connected in turn with the galactose moiety may afford compounds having lower toxicity and better antitumor activity as compared to the parent doxorubicin. Furthermore, this study shows that the α-anomeric configuration of D-galactose rather than β-, and/or a longer length of the spacer (six carbon atoms rather than four in this particular case of **7** and **8**) may result in a more efficacious drug candidates in this series.

4. Experimental

4.1. General methods

Daunorubicin was purchased from the ONOPB factory (Omutninsk, Russia). All reagents and solvents were purchased from Aldrich, Fluka, and Merck. The progress of reactions products, column eluates, and all final samples were analyzed by TLC. TLC was performed on Merck $G60F_{254}$ precoated plates in the following systems: 3:1 petroleum ether–EtOAc (A); 70:10:1 $CHCl_3$–MeOH–HCO_2H (B); 130:60:10:1 $CHCl_3$–MeOH–H_2O–HCO_2H (C). Reaction products were purified by column chromatography on Merck silica gel G60 (0.040–0.063 mm). HPLC analyses were performed on a Shimadzu HPLC LC 10 instrument equipped with a Diasorb C-16 column (4.0 × 250 mm, 7 mc, BioChem Mack, Russia) and variable wavelength UV detector set at 254 nm with an injection volume 10 μL. Elutions were carried out at a flow-rate of 140 μL/min with an 0.01 M 65:35 H_3PO_4–MeCN eluant mixture at 20 °C. The sample concentration was 0.05–0.2 mg/mL. ^1H NMR spectra were recorded on a Varian VXR-400 spectrometer at 400 MHz using the DQ-COSY method. Mass spectra were determined by electrospray ionization (ESI) on a Finnigan MAT 900S spectrometer (Germany, Bremen). For compounds of high molecular weights the data for the predominant monoisotope peak were obtained. All solutions were dried over Na_2SO_4 and evaporated at reduced pressure on a Buchi rotary evaporator at a temperature below 35 °C.

4.2. 3′-*N*-(1-Deoxy-D-glucit-1-yl)-13-(*R*,*S*)-dihydrodaunorubicin (2)

To a stirred solution of daunorubicin hydrochloride (**1**, 140 mg, 0.25 mmol) in a 1:1 mixture of DMF–H_2O (6 mL) D-glucose (1.8 g, 10 mmol) was added. The mixture was stirred at 40 °C for 20 h, and $NaBH_3CN$ (32 mg, 0.5 mmol) was then added. The resulting mixture was stirred for 3 h, an additional amount of $NaBH_3CN$ (32 mg, 0.5 mmol) was then added and the mixture was stirred again at 40 °C for 24 h. The reaction mixture was then diluted with water (50 mL), washed with 10:1 $CHCl_3$–MeOH (20 mL) and extracted with *n*-BuOH (5 × 20 mL). The butanol layers were combined, washed

Table 1
^{1}H NMR spectra of compounds **2**, **3**, **7**, **8**, **16**–**19** (δ ppm)

Compounds								
Chemical shifts	**2**	**3**	**7**	**8**	**16** [b]	**17** [b]	**18**	**19**
Anthracyclinone part								
1	8.08	8.02	8.05	8.02	7.85	8.05	7.90	7.87
2	7.72	7.78	7.73	7.70	7.60	7.72	7.64	7.62
3	7.40	7.46	Nd	7.39	7.85	7.40	7.90	7.90
4-OMe	3.98	3.98			3.97	3.93	3.98	3.97
7	5.42	5.37	5.45	5.40	4.93	5.42	4.95	4.94
8	2.99, 2.26/2.60, 2.42	2.86, 2.22/2.61, 2.35	2.84; 2.54	2.83; 2.51	2.18; 2.15	2.75; 2.50	2.15	2.15
10	3.58, 3.22/3.48, 3.22	3.49, 3.41/3.06, 3.06, J_{gem}18.2	3.58; 3.47	3.56; 3.43	2.97; 2.10	3.52; 3.38	3.43	3.43
13	4.10	4.15						
14	1.65/1.63	1.58/1.56	5.45; 5.38	5.45; 5.39	4.58	5.32	4.57	4.57
Daunosamine part								
1′	5.84	5.82	5.81	5.78	5.32	5.82	5.32	5.32
2′	2.68/2.63	2.70/2.61	5.72; 5.62	2.43	1.95; 2.05	2.78; 2.68	1.85; 1.97	1.97; 2.01
3′	4.25	4.25	4.36	3.74	3.45	4.12	3.39	3.41
4′	Nd [a]	4.69	4.54	4.13	4.12	Nd [a]	3.55	3.65
5′	4.74	4.74	4.72	4.64	4.17	4.45	4.16	4.16
6′	1.53/1.50	1.52/1.50	1.50	1.47	1.20	1.48	1.17	1.19
Polyole part								
1″	3.91/3.72	3.92/3.72, J_{gem}12.7	4.04/3.84	3.50			2.98; 2.93	3.03
2″	4.92	4.50–4.15	5.02	4.66			3.86	3.87
3″	4.70		4.54	4.64–4.44			3.65	3.63
4″	4.25–4.50		4.63				3.09	3.10
5″			4.64–3.96				3.75	3.85
6″	4.23						3.39	3.40
D-Galactose part								
1‴			5.15	5.40	5.57	5.30		
2‴			4.36	4.64–4.44	3.88	4.65		
3‴			4.64–3.96		4.52	4.20		
4‴					4.77	4.68		
5‴				4.54	4.12	4.18		
6‴a				4.36	4.70	4.32		
6‴b				4.36	4.80	4.28		

Spectra for compounds **2**, **3**, **7**, **8**, **16** were recorded in pyridine-d_5+CF_3CO_2D, rt; spectra for compounds **17**–**19** were recorded in Me_2SO-d_6.

[a] Nd—not detected.

[b] ^{1}H NMR parameters for spacers of compounds: **16** (3-methoxy-4-oxybenzyl part): 3.40 (2 H, CH_2), 7.07 (1 H, H-2″), 3.61 (3 H, OMe), 5″ (nd), 6″ (nd); **17** (4-oxybenzyl part): 3.52 (2 H, CH_2), 6.98 (1 H, H-2″), 7.44 (1 H, H-3″), 7.44 (1 H, H-5″), 6.98 (1 H, H-6″).

Table 2
Antitumor activity of compounds **7**, **8**, **17**, and **18** [i.v. injection to mice (BDF_1 C_{57} B1 × DBA_2, males) with P388, 24 h i.p. post implantation of tumor] in comparison with doxorubicin

Compound	Dose (mg/kg)	Injection (i.v.) regimen	Toxic death	ILS (%) [a]
Control			0/10	0
Doxorubicin	7	single	0/6	70
Doxorubicin	14	single	2/6	
7	20	single	0/6	79
7	40	single	0/6	118
7	60	single	1/6	
8	40	single	0/6	65
8	80	single	1/6	
16	40	single	0/6	35
16	60	single	0/6	44
17	40	single	0/6	52
17	60	single	0/6	59
Doxorubicin	2.3	$q2 \times 3$ [b]	4/7	
7	20	$q2 \times 3$ [b]	0/10	79
7	40	$q2 \times 3$ [b]	0/10	133

[a] Increase of lifespan
[b] 24 h post *i.p.* tumor implanting

with concd aq $NaHCO_3$ (30 mL) and water (30 mL) and evaporated. The addition of Et_2O resulted in 150 mg of crude **2** as a dark-red powder, which was put onto column of silica gel and eluted with 80:20:1 $CHCl_3$–MeOH–HCO_2H (100 mL), 2:1 $CHCl_3$–MeOH (100 mL) (to separate the byproduct 13-(*R*,*S*)-dihydrodaunorubicin). Compound **2** was eluated with 13:6:1 $CHCl_3$–MeOH–H_2O. The resulting fractions containing compound **2** were combined, and evaporated to low volume. Addition of *i*-PrOH (15 mL) gave a precipitated solid which was filtered, washed with Et_2O and dried to give **2** as an amorphous red powder (70 mg, 21%), R_f 0.42 (system C), mp 177–178 °C (dec). HR-ESIMS: Calcd for $C_{33}H_{43}NO_{15}$ MW 693.2633. Found: 694.2654 [M+H].

4.3. 3′-*N*-1-(Deoxy-D-galactit-1-yl)-13-(*R*,*S*)-dihydrodaunorubicin (3)

Compound **3** was obtained in a similar manner as **2**, starting from **1** (140 mg, 0.25 mmol) and D-galactose (940 mg, 5 mmol) in 20% yield, R_f 0.42 (system C), mp 186–187 °C (dec), HR-ESIMS: Calcd for $C_{33}H_{43}NO_{15}$ MW 693.2633. Found: 694.2664 [M+H].

4.4. 13-Dimethyl ketal of 14-bromodaunorubicin (4)

Daunorubicin hydrochloride (**1**) (0.7 g, 1.2 mmol) was dissolved in a mixture of MeOH (5 mL), dioxane (2.5 mL), and ethyl orthoformate (2.5 mL) and then Br_2 (0.06 mL) were added. The mixture was stirred for 1 h at 20 °C and afterwards dry K_2CO_3 (0.140 g) was added. The inorganic residue was filtered off quickly and the filtrate evaporated. The resulting crude 13-dimethyl ketal of 14-bromodaunorubicin (**4**) (~0.75 g) was used immediately without purification in the next stage. R_f 0.43 (system B).

4.5. 3′*N*-[-α-D-(Galactopyranosy−(1→6)-*O*-D-1-deoxy-D-glucit-1-yl]doxorubicin (7)

Crude **4** (~1.5 g) was dissolved in MeOH (65 mL). A solution of melibiose (**5**, 3.4 g, 10 mmol) in water (30 mL) was added and the reaction mixture was kept at 40 °C for 4 h, and then $NaBH_3CN$ (0.275 g, 4 mmol) in MeOH (0.5 mL) was added. The mixture was stirred overnight at 37 °C, an additional amount of $NaCNBH_3$ (0.275 g, 4 mmol) in MeOH (0.5 mL) was added, and the mixture was stirred for 24 h. This procedure was repeated twice (16 mmol totally of $NaCNBH_3$ was used) with TLC control. The resulting conjugate **7a** had R_f 0.50 (system C), the starting **4** showed R_f 0.90. Water (200 mL) was added to the reaction mixture and the aqueous solution extracted with $CHCl_3$ (3 × 70 mL). The organic layers were combined, extracted with aq 0.25 N HBr (2 × 50 mL). The dark-red residue that formed between the layers was dissolved in 200 mL of 0.25 N 1:1 HBr–MeOH mixture, and combined with the extracts of the red compound in aq 0.25 N HBr. The combined extracts were incubated during 6 h at 37 °C (to hydrolyze 13-OMe-kethal groups), and after that a solution of HCO_2Na (1.5 g) in water (1 mL) was added to the mixture (pH ~4) to hydrolyze the 14-Br group. The mixture was kept at 37 °C for 24 h under TCL control in the system C. The crude solution of **7** was diluted with water to a volume of 500 mL and combined

with the sorbent XAD-2 swollen in water (~100 mL) and stirred at rt for 6 h until the red color of the solution disappeared. The sorbent was filtered off and washed with water (500 mL). The resulting compound **7** was eluated by a mixture of 1:1:1 *n*-BuOH–Me_2CO–H_2O, evaporated to dryness, and purified by column chromatography (in system C). The resulting fractions containing compound **7** were combined, and evaporated to low volume. Addition of *i*-PrOH (15 mL) gave a precipitate which was filtered off, washed with Et_2O and dried in vacuum to give **7** as amorphous dark-red powder (390 mg, 20%), R_f 0.29 (system C), HPLC Rt 8.62 min, mp 121–123 °C (dec). HR-ESIMS: Calcd for $C_{39}H_{51}NO_{21}$ MW 869.2954. Found: 870.2976 [M+H].

4.6. 3′-*N*-β-D-Galactopyranosyl-(1→4)-*O*-1-deoxy-D-glucit-1-yl-doxorubicin (8)

Compound **8** was obtained by a similar procedure, starting from 1.3 g of **4** and lactose (**6**) in 8% yield. R_f 0.31(system C), HPLC Rt 7.11 min, mp 155–157 °C (dec), HR-ESIMS: Calcd for $C_{39}H_{51}NO_{21}$ MW 869.2954. Found: 870.2981 [M+H].

4.7. 3-Methoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde (12)

To a stirred solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11**) (3.825 g, 9.31 mmol) in dry Me_2CO (30 mL) and dry DMF (20 mL) were added vanillin (**9**, 2.124 g, 13.96 mmol) and K_2CO_3 (2.61 g, 18.61 mmol). The mixture was stirred at 20 °C overnight, and EtOAc (100 mL) was then added. The organic layer was washed sequentially with 10% aq CH_3CO_2H (2 × 100 mL) and H_2O until pH 7 was reached. The organic phase was dried and evaporated. The residue was purified by column chromatography (in system A). Fractions containing compound **12** were combined and evaporated at 35 °C under reduced pressure. The residue was dissolved in petroleum ether (10 mL). On adding Et_2O, the precipitated solid was filtered off and dried to give **12** (2.42 g, 54%), R_f 0.11 (system A), mp 123.5–124.0 °C, $[\alpha]_D^{20}$ −3.5° (*c* 1, MeOH).

^{1}H NMR (Me_2CO-d_6), δ, ppm: 9.93 (1 H, s, –CHO), 7.57 (1 H, dd, $J_{6,5}$ 8.24, $J_{6,2}$ 1.88 Hz, H-6), 7.53 (1 H, d, $J_{2,6}$ 1.88 Hz, H-2), 7.43 (d, 1 H, $J_{5,6}$ 8.24 Hz, H-5), 5.49 (3 H, m, H-1′, H-2′, H-4′), 5.29 (1 H, m, H-3′), 4.48 (1 H, dt, $J_{5',6'}$ 6.45, $J_{5',4'}$ 1.14 Hz, H-5′), 4.22 (2 H, d, $J_{6',5}$ 6.45 Hz, 2 H-6′), 3.93 (3 H; s, OCH_3), 2.20, 2.06, 2.03, 1.97 (4 × 3H, 4 × s, 4 × CH_3CO_2).

4.8. 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-4-oxybenzaldehyde (13)

Compound **13** was obtained as described for **12** starting from tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**11**) and 4-hydroxybenzaldehyde (**10**) in 50% yield, R_f 0.09 (system A), mp 121.5–122.0 °C, $[\alpha]_D^{20}$ −1.1° (*c* 1, MeOH).

^{1}H NMR (Me_2CO-d_6), δ, ppm: 9.98 (1 H, s, –CHO), 7.97 (2 H, d, H-2, H-6), 7.27 (2 H, d, H-3, H-5), 5.64 (1 H, d, $J_{1',2'}$ 7.69 Hz, H-1′), 5.52 (1 H, dd, $J_{4',3'}$ 3.48, $J_{4',5'}$ 1.11 Hz, H-4′), 5.47 (1 H, dd, $J_{2',3'}$ 10.39, $J_{2',1'}$ 7.69 Hz, H-2′), 5.32 (1 H, dd, $J_{3',2'}$ 10.39, $J_{3',4'}$ 3.48 Hz, H-3′), 4.55 (1 H, dt, $J_{5',6'}$ 6.95, $J_{5',4'}$ 1.11 Hz, H-5′), 4.21 (2 H, d, $J_{6',5'}$ 6.39 Hz, 2 H-6′), 2.19, 2.06, 2.04, 1.98 (4 × 3H, 4 × s, 4 × CH_3CO_2).

4.9. 3′-*N*-[3″-Methoxy-4″-*O*-(β-D-galactopyranosyl)-4-oxybenzyl]doxorubicin (16)

To a stirred solution of **4** (~0.75 g) in MeOH (30 mL) was added 3-methoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)hydroxybenzaldehyde (**12**, 1.5 g, 3.11 mmol). The mixture was stirred at 40 °C for 2 h, and $NaBH_3CN$ (0.145 g, 2.3 mmol) was then added. The mixture was stirred overnight at 20 °C, and then additional of $NaBH_3CN$ (0.200 g, 3.2 mmol) was added and the stirring was prolonged for 24 h. The resulting conjugate **14** had R_f 0.55 (system B). The mixture was evaporated at 35 °C under reduced pressure. The residue was dissolved in $CHCl_3$ (70 mL), washed with H_2O (2 × 50 mL). The organic layer was dried and evaporated. The resulted oil was dissolved in a 1:1:1 $CHCl_3$–MeOH–C_6H_6 (45 mL) mixture, evaporated and dried in vacuo, dissolved in dry MeOH (30 mL) and a 0.1 N solution of $NaOCH_3$ (60 mL) was added at 0 °C. The mixture was stirred for 1 h at 20 °C, the resulting compound (**14a**) had R_f 0.05 (system B). Aqueous HBr (0.25 N) was added to the mixture until pH 6 was reached, and then 100 mL of aq 0.25 N HBr was added additionally. The mixture was incubated at 37 °C overnight and then a solution of HCO_2Na (1 g, pH ~4) in H_2O (10 mL) was added. The mixture was kept for 24 h at 37 °C, the desired compound **16** had R_f 0.48 (system C). The mixture was diluted with H_2O to 500 mL and ~100 mL of the sorbent XAD-2 swollen in water was added. The mixture was stirred for 6 h until the red color of the solution disappeared. The sorbent was filtered off and washed with water (~500 mL). Compound **16** was eluted with 1:1:1 *n*-BuOH–Me_2CO–H_2O mixture, the eluate was evaporated. The dry residue was purified by column chromatography (in system C). Fractions containing **16** were combined and evaporated to a volume ~1 mL. Addition of 20 mL of *i*-PrOH gave a precipitate, which was filtered off, washed with Et_2O and dried to give **16** (125 mg, 12%), HPLC Rt 8.62 min, mp 173–174 °C (dec). HR-ESIMS: Calcd for $C_{41}H_{47}NO_{18}$ MW 841.2793. Found: 842.2777 [M+H].

4.10. 3′-*N*-[4″-*O*-(β-D-Galactopyranosyl)-4-oxybenzyl]doxorubicin (17)

Compound **17** was obtained as for **16** in 34% yield; HPLC Rt 8.48 min, mp 170–171 °C (dec), HR-ESIMS: Calcd for $C_{40}H_{45}NO_{17}$ MW 841.2793. Found: 842.2777 [M+H]. HR-ESIMS: $C_{40}H_{45}NO_{17}$ MW Calcd: 811.2687. Found: 812.2699 [M+H].

4.11. 3′-*N*-(D-1-Deoxyglucit-1-yl)doxorubicin (18)

Crude **4** (~0.72 g) was dissolved in MeOH (30 mL). A solution of D-glucopyranose (4.95 g, 27.0 mmol) in water (30 mL) was added and the mixture was kept at 40 °C for 2 h, and $NaBH_3CN$ (0.275 g, 4 mmol) in MeOH (1 mL) was then added and the mixture was stirred overnight at 37 °C. Next, $NaCNBH_3$ (0.275 g, 4 mmol) in MeOH (1 mL) was added and the mixture was stirred at the same temperature for 24 h. This procedure was repeated twice (a total of 16 mmol of $NaCNBH_3$ was added) using TLC monitoring in system C. Water (100 mL) was added to the mixture and the aqueous solution was extracted with $CHCl_3$ (3 × 70 mL). The organic layers were combined, extracted with aq 0.25 N HBr (2 × 50 mL). The acidic aqueous extracts were incubated for 6 h at 37 °C (to hydrolyze the 13-OMe-ketal groups), and then a solution of $NaHCO_2$ (1 g) in water (1 mL) was added to the mixture (to pH ~4.5) to hydrolyze the 14-Br group. The mixture was kept at 37 °C for 24 h under TCL monitoring. The crude solution of **18** was diluted with water to 500 mL and combined with ~100 mL of the sorbent XAD-2 swollen in water and stirred at rt for 6 h until the red color of the solution disappeared. The sorbent was filtered off and washed with water (500 mL). The resulting compound **18** was eluted with 1:1:1 *n*-BuOH–Me_2CO–H_2O mixture and the eluate was evaporated to dryness and purified by column chromatography (system C). The resulting fractions containing compound **18** were combined, and evaporated to a low volume. Addition of *i*-PrOH (15 mL) gave a precipitate, which was filtered off, washed with Et_2O, and dried to give **18** as an amorphous dark-red powder (30 mg, 5%), R_f (system C) 0.24, HPLC Rt 5.25 min, mp 154–155 °C (dec). HR-ESIMS: Calcd for $C_{33}H_{41}NO_{16}$ MW 707.2425. Found: 708.2456 [M+H].

4.12. 3′-*N*-(1-Deoxy-D-galactit-1-yl)doxorubicin (19)

Compound **19** was obtained as for **18**, starting from **4** (0.65 g, 1.1 mmol) and D-galactopyranose (4.95 g, 27 mmol) in 5% yield, R_f (system C) 0.44, HPLC Rt 5.25 min, mp 175–176 °C (dec) HR-ESIMS: Calcd for $C_{33}H_{41}NO_{16}$ MW 707.2425. Found: 708.2460 [M+H].

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References

1. Garnier-Suillerot, A.; Marbeuf-fueye, C.; Salerno, M.; Loetchutinat, C.; Fokt, I.; Krawczyk, M.; Kowalczyk, T.; Priebe, W. *Curr. Med. Chem.* **2001**, *8*, 51–64.
2. Mazerska, Z.; Woynarowska, B.; Stefanska, B.; Borowski, E.; Martelli, S. Antitumor activity of new N-substituted daunorubicin derivatives. *Drugs Exp. Clin. Res.* **1987**, *13* (6), 345–351.
3. Arcamone, F.; Animati, F; Bigioni, M; Capranico, G; Cacerrini, C.; Cipollone, A.; De Cesare, M.; Ettore, A.; Guano, F.; Manzini, S.; Monteagudo, E.; Pratesi, G.; Salvatore, C.; Supino, R.; Zunino, F. *Biochem. Pharmacol.* **1999**, *57*, 1133–1139.
4. Zunino, F.; Prateshi, G.; Perego, P. *Biochem. Pharmacol.* **2001**, *61*, 933–938.
5. Ghosh, A.; Khan, S.; Marini, F.; Nelson, J. A.; Farquhar, D. *Tetrahedron Lett.* **2000**, *41*, 4871–4874.
6. Houba, P. H. J.; Boven, E.; Erkelens, C. A. M.; Leenders, R.g.g.; Scheren, J. W.; Pinedo, H. M.; Haisma, H. J. *Br. J. Cancer* **1998**, *78* (12), 1600–1606.
7. Barondes, S. H.; Castronovo, V.; Cooper, D. N. W.; Cummings, R. D.; Drickamer, K.; Feizi, T.; Gitt, M. A.; Hirabayashi, J.; Hudges, C.; Kasai, K.-I.; Leffler, H.; Liu, F.-T.; Lotan, R.; Mercurio, A. M.; Monsigny, M.; Pillai, S.; Poirer, F.; Raz, A.; Rigby, P. W. J.; Rini, J. M.; Wang, J. L. *Cell* **1994**, *76*, 597–598.
8. Lotan, R.; Ito, H.; Yasui, W.; Yokozaki, H.; Lotan, D.; Tahara, E. *Int. J. Cancer* **1994**, *56*, 474–480.
9. Povarov, L. S.; Isayeva, N. I.; Rubasheva, L. M.; Olsufyeva, E. N. *Russ. J. Org. Chem.* **1979**, *15*, 1560–1561 (Russ).
10. Olsufyeva, E. N.; Rosynov, B. V. *Soviet J. Bioorg. Chem.* **1991**, *16* (4), 476–481.
11. Gray, G. R. *Arch. Biochem. Biophys.* **1974**, *163* (1), 426–428.