Synthesis of daunorubicin conjugates with fragments of H type 5, Le^a, Le^x antigens and *N*-fucoglycan

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Daunorubicin was conjugated with spacered *N*-glycosides of di- and trisaccharides containing terminal α-L-fucopyranose residues. The synthesis was carried out by *N*-monoalkylation of daunorubicin hydrochloride (**1**) in aqueous DMF in the presence of $NaHCO_3$ using *N*-β-glycoside *N*-bromoacetylglycyl derivatives, namely, α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-NHCOCH$_2$NHCOCH$_2$Br (**2a**), α-L-Fuc*p*-(1→4)-[β-D-Gal*p*-(1→3)]-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**3a**), α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**4a**), and α-L-Fuc*p*-(1→6)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**5a**). The conjugates of daunorubicin were obtained as hydrochlorides (**2b—5b**) in 40% yield. Their oligosaccharide components are fragments of H type 5, Le^a, Le^x antigens and *N*-fucolglycan, respectively. 0(I) Erythrocytes hemagglutination inhibition assay was performed with conjugates **2b—5b** and *Ulex europaeus* (UEA 1), *Lotus tetragonolobus* (LTA), and *Laburnum anagyroides* (LAA) plant fucolectins.

Key words: glycoconjugates, daunorubicin, *N*-alkylation, α-L-fucose, oligosaccharides, *N*-glycosides, fucoantigens, H type 5, Le^a, Le^x, fucolectins, *Ulex europaeus*, *Lotus tetragonolobus, Laburnum anagyroides*.

Glycosylated anthracycline quinone antibiotics daunorubicin (**1**) and doxorubicin are widely used in cancer chemotherapy. An essential disadvantage of these drugs is significant cardiotoxicity, as well as the resistance of some tumors to their action. One way to solve these problems is to chemically modify the indicated compounds with carbohydrates at the hydroxy or amino group of the monosaccharide residue, α-L-daunosamine. The glycosylation of the hydroxyl group of α-L-daunosamine in daunorubicin with 2-, 6-deoxy- or 2,6-dideoxymonosaccharide derivatives leads to the analogs of the third generation of anthracycline antibiotics (disaccharide-containing anthracyclines). In this case, compounds with α-2,6-dideoxymonosaccharide moieties show the highest cytotoxicity.[1,2] The modification of antibiotics at the amino group was carried out by the condensation with mono- and disaccharides potentially containing an aldehyde group,[3] as well as with various polysaccharides after the introduction of aldehyde groups by periodate oxidation (see Ref. 4 and references cited therein), followed, as a rule, by the reduction of the Schiff base with $NaBH_3CN$. The carbohydrate can also be attached to the amino group of daunorubicin during *N*-acylation, for example, using a special spacer allowing the starting antibiotic to be regenerated from the glycoconjugate (synthesis of the so-called prodrugs, see Ref. 5 and references cited therein).

The binding of a carbohydrate-modified antibiotic to a tumor cell can be achieved through a carbohydrate-protein interaction, which involves numerous proteins having carbohydrate-binding sites, the enzymes of carbohydrate biosynthesis and metabolism, in particular, glycosyltransferases, kinases, epimerases, reductases, glycosidases, and carbohydrate-binding proteins, lectins.[6] The latter are present on the surface of both normal[7] and tumor human cells.[8] The biochemical processes accompanying the malignant transformation of cells are to a large extent associated with a change in the carbohydrate composition,[9] with L-fucose being one of the main carbohydrate markers of oncogenesis.[10] A sharp increase in α-L-fucosylation is one of the indications of malignant transformation of cells and contributes to the development of various processes accompanying oncogenesis: cell proliferation, angiogenesis, metastasis, and suppression of the immune system. The entire biochemistry of the organism associated with L-fucose changes: the synthesis of GDP-fucose increases, the α-fucosidase activity changes and the α-fucosyltransferase activity increases, the fucosylation of the hydroxy group at the C(6) atom of the core residue of

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N-acetylglucosamine in *N*-glycoproteins increases, and the ratio of fucoantigens changes.[11]

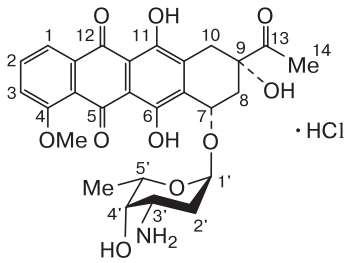
This work is devoted to the synthesis of glycoconjugates of antibiotic daunorubicin containing terminal α-L-fucopyranose moieties attached to different hydroxy groups of the neighboring monosaccharide residue in the composition of spacered *N*-glycosides of di- or trisaccharides, the fragments of important fucoantigens. The synthesized earlier[12] *N*-(*N*-bromoacetylglycyl)-β-glycopyranosylamines of di- and trisaccharides, namely, α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-NHCOCH$_2$NHCOCH$_2$Br (**2a**), α-L-Fuc*p*-(1→4)-[β-D-Gal*p*-(1→3)]-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**3a**), α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**4a**), and α-L-Fuc*p*-(1→6)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$Br (**5a**), were used as the starting compounds for the introduction of fucoantigens into daunorubicin. Compounds **2a**—**4a** are the spacered fragments of the glucoanalog of H antigen, Le^a antigen, and Le^x antigen, respectively, while compound **5a** is the *N*-fucoglycan, a fragment of the *N*-glycoprotein carbohydrate-peptide bond region, in which the asparagine residue is replaced with a glycine moiety.

Results and Discussion

Earlier, *N*-bromoacetyl derivatives of glycopyranosylamines of mono- and oligosaccharides[13,14] were used to obtain glycoconjugates of physiologically active compounds or proteins using *N*- or *S*-alkylation (see Refs 14, 15 and references cited therein), but bromoacetylglycyl derivatives with terminal α-L-fucose **2a—5a** are used for this purpose for the first time. The choice of these *N*-glycosides is substantiated by the detection of receptors of fucose-containing compounds on the surface of tumor cells in patients with leukemia,[16] as well as by the widespread application of the antibiotic daunorubicin for the treatment of leukemia.[17] The introduction of these glycosides into daunorubicin has a chance of increasing the selectivity of antibiotic delivery to cancer cells due to carbohydrate-protein interactions, as shown in the work:[18] polymethacrylamide was independently modified with peptide-spacered doxorubicin and 2-aminofucose, due to which the conjugate was delivered to cancer cells with subsequent enzymatic (lysosomes) regeneration of the antibiotic (macromolecular prodrug). Daunorubicin-based fucoglycoconjugates earlier have not been synthesized; only one study[19] used L-fucose and daunorubicin-containing liposomes.

The earlier[13] synthesized *N*-bromoacetyl derivative of lactose β-glycosylamine, β-D-Gal*p*-(1→4)-β-D-Glc*p*-NHCOCH$_2$Br (**6a**), was used to select the conditions for *N*-monoalkylation of daunorubicin hydrochloride. The study resulted in the following conditions for the synthesis of glycoconjugates **2b—6b**: aqueous DMF, $NaHCO_3$, **1** : **2a—6a** = 1 : 1. The glycoconjugates were isolated as hydrochlorides using reverse phase chromatography on C_{18}-modified silica gel in aqueous MeOH with HCl additives. The composition of the colored fractions eluted from the column was analyzed by high-resolution mass spectrometry, which showed that daunorubicin was eluted first, then *N*-monoalkylated daunorubicin (the major product) and *N*,*N*-dialkylated daunorubicin (the minor product). The repeated chromatography under the same conditions of fractions with *N*-monoalkylated daunorubicin gave red powders of pure glycoconjugates **2b—6b** in the form of storage-stable hydrochlorides in ~40% yield (Scheme 1).

Scheme 1



1

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-NHCOCH$_2$NHCOCH$_2$-X

2a,b

α-L-Fuc*p*-(1→4)-[β-D-Gal*p*-(1→3)]-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$-X

3a,b

α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$-X

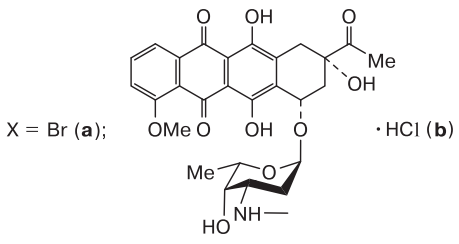
4a,b

α-L-Fuc*p*-(1→6)-β-D-Glc*p*NAc-NHCOCH$_2$NHCOCH$_2$-X

5a,b

β-D-Gal*p*-(1→4)-β-D-Glc*p*-NHCOCH$_2$-X

6a,b



1 + **2a—6a** $\xrightarrow{i,\ ii}$ **2b—6b**

Reagents and conditions: *i*. $NaHCO_3$, DMF—H_2O (3 : 2), 20 °C, 6 h; *ii*. HCl.

The composition of conjugates **2b—6b** was confirmed by high resolution mass spectrometry. It should be noted that in the mass spectra of negative ions, conjugates