NEW SEMISYNTHETIC ANTHRACYCLINE GLYCOSIDES

Josef V. JIZBA, Petr SEDMERA, Věra PŘIKRYLOVÁ, Jindřich VOKOUN, Karel MIKULÍK and Zdenko VANĚK

Institute of Microbiology, Czechoslovak Academy of Sciences, 142 20 Prague 4

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3-Trifluoroacetamido-4-O-trifluoroacetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (*VII*) was coupled under Koenigs–Knorr glycosidation conditions to (7*S*)-O-(2-hydroxyethyl)daunomycinone (*IV*), (7*R*)-O-(2-hydroxyethyl)daunomycinone (*V*), and (7*S*)-O-(4-hydroxy-2-butynyl)-daunomycinone (*VI*). The deprotection of isolated N-trifluoroacetyl derivatives of α- and β--glycosides *XVI*, *XVII*, *XX*, *XXI*, and *XXIV* yielded free glycosides *XVIII*, *XIX*, *XXII*, *XXIII*, and *XXV*. Their anomeric configuration was determined by NMR spectroscopy. Reaction of 3,4-di-O-acetyl-2,6-dideoxy-α,β-L-arabinopyranosyl chloride (*II*) with *IV* gave glycosides *XII* and *XIII*. Their deacetylation provided free glycosides *XIV* and *XV*. An analogueous reaction with 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (*III*) with *IV* lead to the N-acetyl glycoside *IX* only.

Anthracyclines represent a relatively large group of natural, semisynthetic, and synthetic compounds[1-3]. Some compounds of this type are used in cancer treatment, several promissing candidates are in clinical testing[4-6]. Intense work on new anthracyclines involve the modification of both aglycone and sugar moiety. The changes of saccharide part are achieved by substitution of existing groups (N-alkylation, N-acylation, O-alkylation, formation of tetrahydropyranyl derivatives, etc.), changes in configuration of some substituents, deamination or even by an attachment of sugar residues not encountered in natural anthracyclines (mono- and oligosaccharidic), existing in pyranose or furanose forms. However, in all cases mentioned the sugar residues are connected to the secondary hydroxyl group of the aglycone alicyclic ring. Reaction of daunomycinone (*I*) with diols providing its 7-O-hydroxyalkyl derivatives[7] opens a way to a new type of glycosides with sugar part bonded to the primary hydroxyl group of the new side chain. This work describes the preparation and properties of glycosides of 2,6-dideoxy-α,β-L-arabinopyranose (2-deoxyrhamnose) and 3-amino-2,3,6-trideoxy-α,β-L-lyxopyranose (daunosamine) derived from modified aglycones of the daunomycinone type.

Two main approaches for coupling of sugar to aglycone are most often found in the literature: reaction of a glycal with aglycone, catalyzed by an acid[8-12] and Koenigs–Knorr reaction starting with a protected glycosyl halogenide. As a catalyst in the latter method is used a mixture of mercuric oxide, mercuric bromide, and

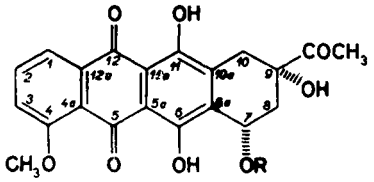
molecular sieve (3–4 Å)[13–15], mixture of mercuric cyanide, mercuric bromide, and molecular sieve[17] or silver trifluoromethyl sulfonate[18–20]. Also described are catalyses by a mixture of trifluoromethanesulfonic acid anhydride, tetrabutylamonium bromide, and 2,4-colidine at low temperature[21,22] or by tin(IV) chloride[23,24]. A mixture of both anomers is obtained in most cases. The reaction is usually performed in dichloromethane[13] or tetrahydrofuran[25]. The deprotection is achieved by standing in methanolic sodium methoxide or aqueous sodium hydroxide solution.

We obtained the best results in the reaction of crude 3,4-di-O-acetyl-2,6-dideoxy-α,β-L-arabinopyranosyl chloride (*II*) and 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (*III*) with (7*S*)-O-(2-hydroxyethyl)daunomycinone (*IV*) in chloroform (used for better solubility) employing the mixture of mercuric bromide, mercuric cyanide, and Potasit 3 Å* or by a mixture of mercuric bromide, mercuric oxide, and Potasit 3 Å. In the glycosidation of *IV*, its (7*R*)-analogue *V* and (7*S*)-O-(4-hydroxy-2-butynyl)daunomycinone (*VI*) with 3-trifluoroacetamido-4-O-trifluoroacetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (*VII*), it was necessary to add mercuric cyanide to the mixture of mercuric oxide, mercuric bromide, and Potasit 3 Å. The reaction mixture containing α- and β- forms of protected glycoside, starting aglycone, and unreacted glycosyl chloride was separated chromatographically. As expected, the α-anomers prevailed. N-trifluoroacetyl derivatives *XVI*, *XVII*, *XX*, *XXI*, and *XXIV* were isolated in reactions of *VII*.

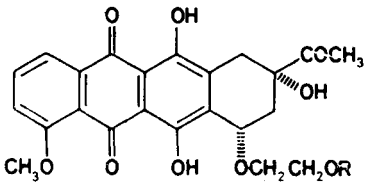
The site of sugar attachment to aglycone was determined by NMR spectroscopy. Negligible changes in chemical shifts of atoms in the vicinity of C-9 allow to exclude this position. Signals of phenolic hydroxyl group protons at C-6 and C-11 observed in ^{1}H NMR spectra (Table I) leave the terminal primary hydroxyl group of the side chain at C-7 as the last possibility. A strong evidence for glycosidation at this position is the downfield shift of signals of the corresponding terminal carbon atoms in ^{13}C NMR spectra with respect to the starting aglycone. The configuration of the anomeric center of the sugar was determined either from the width of the H-1′ signal (5–6 Hz for an equatorial, 8–13 Hz for an axial proton) or from the magnitude of the direct coupling constant $^1J(^{13}C, ^1H)$ of carbon C-1′ (169–173 Hz for α-, 160–164 Hz for the β-configuration)[26,27]. Chemical shift of carbon C-1′ (with hexopyranoses always larger in the β-series) was used as a supplementary criterion.

Only one product (*VIII*) was isolated from the reaction of *IV* with crude 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α,β-lyxopyranosyl chloride (*III*). Its deprotection provided the glycoside *IX*, containing according to ^{1}H NMR a N-acetyl group and assigned to the α-series on the basis of the H-1′ signal width. When this reaction was performed in tetrahydrofuran in which the aglycone *IV* is excelently soluble, an another product was isolated. However, its ^{1}H NMR spectrum did not contained neither signals of acetyl groups or the doublet of the secondary methyl C-6 of the

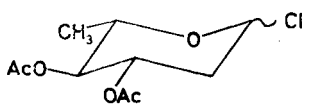
* 1 Å = 10^{-10} m.



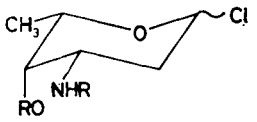
I, R = H
XXVI, R = α-Y; R' = R" = H



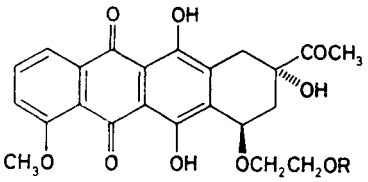
IV, R = H
VIII, R = α-Y; R' = R" = Ac
IX, R = α-Y; R' = Ac; R" = H
X, R = (*R*)-X
XI, R = (*S*)-X
XII, R = α-Z; R' = Ac
XIII, R = β-Z; R' = Ac
XIV, R = α-Z; R' = H
XV, R = β-Z; R' = H
XVI, R = α-Y; R' = CF_3CO; R" = H
XVII, R = β-Y; R' = CF_3CO; R" = H
XVIII, R = α-Y; R' = R" = H
XIX, R = β-Y; R' = R" = H



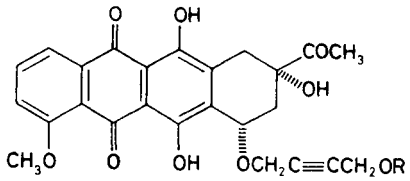
II



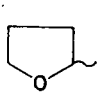
III, R = CH_3CO
VII, R = CF_3CO



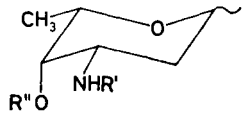
V, R = H
XX, R = α-Y; R' = CF_3CO; R" = H
XXI, R = β-Y; R' = CF_3CO; R" = H
XXII, R = α-Y; R' = R" = H
XXIII, R = β-Y; R' = R" = H



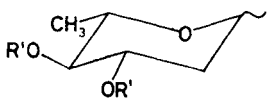
VI, R = H
XXIV, R = α-Y; R' = CF_3CO; R" = H
XXV, R = α-Y; R' = R" = H



X



Y



Z

sugar residue. Mass spectrum gave a molecular ion m/z 512, $C_{27}H_{28}O_{10}$ (Table III). The interpretation of ^{13}C NMR spectrum reveals that all signals in addition to those of the aglycone are doubled and form two series: OCHO, OCH_2, and CH_2CH_2. That leads to the conclusion that the products of this reaction are tetrahydrofuryl derivatives *X*, *XI* of aglycone *IV*. Koenigs–Knorr reaction of 3,4-di-O-acetyl-2,6--dideoxy-α,β-L-arabinopyranosyl chloride (*II*) with *IV* in chloroform yielded two protected glycosides *XII* and *XIII* assigned according to the magnitude of ^1J(C-1′, H-1′) (Table II) to the α- and β- series. This conclusion was confirmed by multiplicity of H-1′ proton signal in ^1H NMR spectra of deprotected glycosides *XIV* and *XV*. Coupling of *VII* to the aglycone *IV* provided a mixture of glycosides isolated as N-trifluoroacetyl derivatives *XVI* (α-) and *XVII* (β-) (see Tables I and II). Free glycosides *XVIII* and *XIX* were prepared by their deprotection. Reaction of *VII* with aglycone *V* gave the α-glycoside *XX* and a small amount of β-glycoside *XXI* (Table I). After deprotection, *XX* gave free glycoside *XXII* and *XXI* gave free *XXIII*. With aglycone *VI*, the only isolated product was the protected glycoside *XXIV*. Multiplets of H-1′ and H-7 in ^1H NMR spectra both *XXIV* and the free glycoside *XXV* overlap. Since both multiplets are narrow, these compounds probably belong to the α-series.

Compounds *VIII*, *XII*, *XIII*, *XVIII*, *XX* and *XXIII* inhibited growth of *Bacillus subtilis* in an orientation agar diffusion test.

EXPERIMENTAL

Melting points were determined in a Kofler apparatus. Optical rotations were measured in chloroform and methanol using a Bendix–Ericson ETL 143A instrument. ^1H and ^{13}C NMR spectra were studied with a Jeol FX-60 FT NMR spectrometer (FT mode, 59·797 and 15·036 MHz) at 25 °C. Chemical shifts are given in the δ-scale (±0·005 and ±0·06 ppm). Multiplicity of ^{13}C NMR signals was determined by off-resonance decoupling experiments. Direct coupling constants $^1J(^{13}C, ^1H)$ were measured in proton-coupled spectra obtained by the gated decoupling method (decoupler off during the acquisition). Mass spectra were measured using a Varian MAT-311 instrument (70 eV, ionizing current 1 mA, ion source temperature 200°C, direct inlet temperature T_d given with the individual spectra in Tables III and IV). High-resolution measurements (±3 ppm) were performed by a "peak-matching" technique with perfluorokerosene as a standard. Sephadex LH-20 (Pharmacia, Sweden) and silica gel (Herrmann, F.R.G.) were used for column chromatography. Ready-made plates Silufol R[20] (Kavalier, Czechoslovakia) or Kieselgel F-60 (Merck, F.R.G.) were used for preparative thin-layer chromatography. Following chromatographic systems were used: chloroform–benzene–ethyl acetate–methanol 7 : 7 : 3 : 1·5 (S1), chloroform–hexane — 7 : 3 (S2), hexane–chloroform–methanol 5 : 2 : 0·7 (S3), chloroform–hexane 9 : 1 (S4) and 3 : 7 (S5) and chloroform–methanol–water–26% aqueous ammonium hydroxide 20 : 3 : 0·1 : 0·01 (S6). 3-Amino-2,3,6-trideoxy-α-L-lyxopyranose (daunosamine) was synthetized from L-rhamnose according to ref.[28]. Chloride of its pertrifluoroacetyl derivative (*III*) was prepared following the known procedure[29]. Starting 2,6-dideoxy-α,β-L-arabinopyranose (2-deoxyrhamnose) was obtained from L-rhamnose by Iselin–Reichstein method[29]. The protection of hydroxyl groups by acetylation and the preparation of glycosyl chlorides were performed according to ref.[13]. The preparation of aglycones *IV*— *VI* was described earlier[7].

TABLE I

Proton chemical shifts and coupling constants (Hz, in parentheses) in some anthracyclinone

Compound Solvent[b]	Proton[a]						
	H-1	H-2	H-3	4-OCH_3	6-OH	11-OH	H-7
VIII C	8·03 dd (7·3, 1·2)	7·76 dd (7·3, 7·3)	7·39 dd (7·3, 1·2)	4·08 s	13·92 s	13·27 s	5·12 mt
IX A	8·04 dd (7·3, 2·4)	7·79 dd (7·3, 7·3)	7·41 dd (7·3, 2·4)	4·08 s	—	—	5·08 mt
XII A	8·03 dd (7·8, 1·5)	7·75 dd (7·8, 7·8)	7·37 dd (7·8, 1·5)	4·08 s	13·92 s	13·25 s	4·70—5·40 mt
XIII A	8·03 dd (7·7, 1·8)	7·75 dd (7·7, 7·3)	7·37 dd (7·3, 1·8)	4·08 s	13·94 s	13·27 s	4·47—5·28 mt
XIV A		7·17—8·08 mt		4·08 s	13·92 s	13·27 s	5·10 dd (2·4, 3·7)
XV A		7·23—8·08 mt		4·08 s	13·92 s	13·27 s	5·08 mt $W = 6·8$
XVI B	8·06 dd (7·3, 2·4)	7·78 t (7·3)	7·41 dd (7·3, 2·4)	4·08 s	—	—	5·08 mt $W = 7·3$
XVII B		7·25—8·17 mt		4·08 s	13·90 s	13·31 s	5·06 mt $W = 6·1$
XVIII B		7·25—8·06 mt		4·06 s	—	—	5·04 mt
XIX D		7·24—8·08 mt		4·06 s	—	—	5·96 mt
XX A	8·06 dd (7·3, 2·0)	7·76 t (7·3)	7·37 dd (7·3, 2·0)	4·08 s	13·90 s	13·31 s	5·12 t (4·9)
XXI A	8·04 dd (7·9, 1·2)	7·17 t (7·9)	7·37 dd (7·9, 1·2)	4·10 s	13·90 s	13·33 s	5·07 dd (4·9, 3·7)
XXII D		7·15—7·72 mt		3·88 s	—	—	5·34 mt
XXIV A	8·02 dd (7·9, 1·2)	7·76 t (7·9)	7·37 dd (7·9, 1·2)	4·07 s	13·83 s	13·25 s	5·22 mt
XXV B		7·25—8·11 mt		4·08 s			5·06—5·21 mt

[a] Signal assignment is based on double resonance experiments and comparison with similar

TABLE I
analogues

Proton[a]					
H-8*a*	H-8*e*	H-10*a*	H-10*e*	H-1′	H-6′
		2·96 d (19·5)	3·21 dd (19·5, 0·8)	5·12 mt	1·09 d (6·1)
		2·99 d (19·2)	3·20 dd (19·2, 1·2)	4·86 mt $W = 6·1$	1·20 d (6·4)
		2·92 d (18·9)	3·21 dd (18·9, 1·0)	4·70— 5·40 mt	1·15 d (6·1)
		2·95 d (19·5)	3·21 dd (19·5, 0·9)	4·47— 5·18 mt	1·18 d (6·1)
1·96 dd (14·7, 3·7)	2·44 ddd (14·7, 2·4, 1·0)	2·93 d (19·5)	3·24 dd (19·5, 1·0)	4·82 dd (1·2, 2·4)	1·26 d (6·1)
				4·54 dd (1·8, 8·6)	1·28 d (6·1)
		2·98 d (19·5)	3·23 dd (19·5, 0·8)	4·88 mt $W = 6·1$	1·20 d (7·3)
1·94 dd (14·7), 3·6)		2·99 d (19·0)	3·24 dd (19·0, 1·0)	4·52 dd (9·3, 2·1)	1·16 d (7·3)
				5·04 mt	0·95 d (6·1)
				5·96 mt	1·22 d (6·1)
2·58 d (14·8, 4·9)	2·13 ddd (14·8, 4·9, 0·8)	3·30 d (18·6)	2·97 dd (18·6, 0·8)	4·88 mt $W = 6·1$	1·15 d (6·1)
2·56 dd (14·7, 3·7)	2·11 ddd (14·7, 4·9, 2·2)	3·37 d (17·1)	2·94 dd (17·1, 1·2)	4·49 dd (9·8, 2·4)	1·32 d (6·1)
				5·34 mt	1·08 d (7·3)
2·01 dd (14·6, 3·7)	2·48 ddd (14·6, 2·4, 1·0)	2·92 d (18·9)	3·24 dd (18·9, 1·0)	5·22 mt	1·27 d (6·7)
		2·93 d (19·5)	3·23 dd (19·5, 1·0)	5·06— 5·21 mt	1·38 d (6·1)

compounds; [b] A $CDCl_3$, B $CDCl_3$ + CD_3OD 4 : 1, C $CDCl_3$ + CD_3OD 3 : 1, D CD_3OD.

TABLE II

^{13}C Chemical shifts in some anthracyclinone analogues

Carbon[b]	Compound/Solvent[a]							
	VIII[c] C	*X, XI* A	*XII*[c] A	*XIII*[c] A	*XIV* A	*XV* A	*XVI* B	*XVII* B
1	118·5	118·4	118·4	118·3	118·4	118·4	118·5	118·7
2	135·8	135·5	135·7	135·2	135·7	135·7	135·8	135·8
3	119·8	119·7	119·8	119·8	119·8	119·8	119·8	119·4
4	161·0	160·9	161·0	161·0	161·0	161·0	161·1	161·1
5	186·7[f]	186·7	186·7[f]	186·4[f]	186·7[f]	186·5[f]	186·5[f]	186·9[f]
6	155·6[g]	155·8[f]	155·9[g]	155·9[g]	155·9[g]	155·9[g]	156·1[g]	155·6[g]
7	71·0[h]	69·1	69·3[h]	70·6[h]	69·3[h]	71·7[h]	69·4[h]	71·6[h]
8	33·5	33·5	33·8	33·7	33·8	33·7	33·8	33·5
9	76·7	76·5	73·8	74·2	77·7	77·6	76·3	77·2
10	31·8	32·4	32·0	32·5	32·0	32·4	32·1	31·7
11	156·1[g]	156·5[f]	156·5[g]	156·4[g]	156·5[g]	156·2[g]	156·1[g]	156·1[g]
12	186·9[f]	186·7	187·0[f]	186·7[f]	187·1[f]	186·9[f]	186·7[f]	186·9[f]
13	213·5	213·2	213·1	213·0	212·7	212·9	213·2	212·9
14	24·8	24·8	25·0	25·0	25·0	25·0	25·0	25·0
4a	121·0	121·0	122·5	n.o.	n.o.	n.o.	121·2	n.o.
5a	111·5	111·2	111·4	111·4[j]	111·4[j]	111·2	111·5[j]	111·5[j]
6a	133·7[i]	134·0[g]	133·9[i]	133·9[i]	133·7[i]	133·6[i]	133·6[i]	133·9[i]
10a	134·1[i]	135·0[g]	135·2[i]	135·2[i]	135·0[i]	133·9[i]	134·8[i]	134·6[i]
11a	111·5	111·2	111·4	113·4[j]	111·6[j]	111·2	111·5[j]	111·5[j]
12a	134·9[i]	135·4[g]	135·5[i]	134·4[i]	136·6[i]	135·4[i]	135·9[i]	135·9[i]
CH_3O	56·7	56·7	56·7	56·7	56·7	56·7	56·8	56·7
α	69·3	67·1	69·3	69·5	69·3	69·7	69·4	69·0
β	66·8	66·3	66·7	68·0	66·0	67·8	66·8	68·2
1′	97·5		97·2[d]	99·0[d]	97·5	99·4	97·1[d]	99·3[d]
2′	29·8	104·0[e]	35·1	36·4	34·4	39·0	29·2	31·1
3′	43·8	31·7[e]	65·8[h]	69·4[h]	67·7[h]	69·4[h]	66·0[h]	67·4[h]
4′	65·2[h]	23·4[e]	68·9[h]	69·5[h]	67·7[h]	69·4[h]	66·0[h]	67·4[h]
5′	69·3[h]	69·5[e]	69·3[h]	70·0[h]	69·3[h]	71·6[h]	68·6[h]	69·4[h]
6′	16·8		17·5	17·5	16·7	17·5	16·6	16·5

[a] A $CDCl_3$, B $CDCl_3$ + CD_3OD 4 : 1, C $CDCl_3$ + CD_3OD 3 : 1, D CD_3OD; [b] Signal assignment is based on the chemical shift consideration, signal multiplicity and comparison with related compounds. Signals in the same column bearing the same subscript can be interchanged; [c] additional signals *VIII*: 20·7 q, 22·6 q, 170·6 s, 171·1 s; *XII*: 20·9 q, 170·2 s; *XIII*: 20·8 q, 170·1 s;

TABLE II
(*Continued*)

Compound/Solvent[a]						
XVIII B	*XIX* D	*XX* D	*XXI* A	*XXIII* D	*XXIV*[c] A	*XXV*[c] B
118·6	120·3	120·2[k]	118·4	120·5	118·4	118·4
135·8	135·1	137·4	135·2	137·4	135·7	135·2
119·8	120·3	121·1[k]	119·8	120·5	119·7	119·7
161·0	162·2	162·2	160·3	162·4	161·0	161·0
186·7[f]	187·3[f]	186·5[f]	n.o.	187·8[f]	186·7[f]	186·9
155·4[g]	156·1[g]	155·7[g]	155·2[f]	156·1[g]	155·7[g]	155·8[f]
69·5[h]	73·2[h]	71·9[h]	71·2[g]	71·6[h]	69·1[h]	70·7[g]
33·7[k]	33·7	33·3	38·6	38·6	33·7	33·7
75·8	77·7	76·3	76·4	79·8	76·7	76·7
32·5	33·4	38·2	31·7[g]	33·4[j]	32·8	33·3
156·1[h]	157·1[g]	157·5[h]	156·5[f]	157·7[g]	156·2[g]	156·2[f]
187·0[g]	187·5[f]	187·7[g]	n.o.	187·8[f]	186·7[f]	186·9
213·1	215·2	211·0	n.o.	211·0	212·5	212·5
25·0	25·1	24·6	24·6	24·6	24·8	25·0
120·9	121·0	122·8	n.o.	121·5	122·8	123·8
111·5[j]	112·0[j]	112·2[j]	111·6	112·4	133·5[i]	111·4
133·7[i]	135·1[i]	135·8[i]	135·9[h]	131·6[i]	133·5[i]	133·6[h]
134·8[i]	135·1[j]	135·8[i]	135·9[h]	136·3[i]	135·0[i]	135·0[h]
111·5[j]	112·0[j]	112·2[j]	111·6	112·4	111·5	111·4
135·5[i]	137·0[i]	138·0[i]	136·2[h]	138·3[i]	135·4[i]	135·4[h]
56·8	57·2	57·1	56·7	57·3	56·5	56·7
69·5	67·8	71·6	69·4	72·0	54·3	54·5
66·4	62·4	68·1	69·4	68·1	58·2	58·7
97·1	97·6	98·5	100·2	98·9	94·5	95·9
32·2[k]	32·9	30·0	31·5[i]	34·4[j]	29·2	32·2
49·4	49·5	48·3	49·1	49·1	46·3	46·4
66·1[h]	67·2[h]	67·7[h]	68·5[h]	68·1[h]	66·1[h]	66·4[h]
67·4[h]	70·7[h]	69·4[h]	68·7[h]	70·4[h]	68·7[h]	69·1[h]
16·8	17·0	17·3	16·6	17·5	16·5	16·9

XXIV: 82·1 s, 82·7 s; *XXV*: 82·0 s, 82·9 s; [d] direct couplings ^1J [Hz]: *XII*: 169·9; *XIII*: 161·1; *XVI*: 172·0; *XVII*: 164·1; [e] minor component: 32·0 t, 69·8 t, 104·2 d; [f–k] values in the same column and bearing the identical superscript may be interchanged.

(7*S*)-O-(2′-O-(3″-acetamido-4″-O-acetyl-2″,3″,6″-trideoxy-α-L-lyxopyranosyloxy)ethyl)daunomycinone (*VIII*)

Mercuric bromide (40 mg), mercuric oxide (35 mg), and Potasit 3 Å (5 g) were added to the solution of aglycone *IV* (150 mg, 0·34 mmol) in chloroform (50 ml). Crude 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (4 × 60 mg, 0·95 mmol) was stepwise added

TABLE III

Mass spectra of selected compounds

	VIII[a]		*X*[b], *XI*[b]		*XII*[c]		*XIII*[d]
	m/z	%	*m/z*	%	*m/z*	%	%
M^+	655	—	512	0·1[e]	656	0·005	0·01
M − 43	*	—		—	613	0·01	0·02
Aglycone fragmentation[30]							
	442	—		0·1[e]		0·04	—
	382	—		—		—	0·5[e]
	362	32[e]		1[e]		0·6[e]	17[e]
	344	24[e]		9[e]		18[e]	11[e]
	339	6[e]		6[e]		10[e]	2[e]
	337	2		3[e]		2[e]	2[e]
	329	2		1[e]		2[e]	2[e]
	321	1		1[e]		2[e]	3[e]
	319	1		1[e]		2[e]	2[e]
	309	1		1[e]		—	—
	301	15[e]		3[e]		5[e]	6[e]
Sugar fragmentation[31]							
C_1	214	14[e]	104	3[g]	215	0·8[e]	1[e]
	172	11[f]	101	13[h]	172	3	4
D_2	143	22[e]	87	9[i]	155	9	10
D_2'	101	42[e]	71	100[j]	144	3	3
C_2	95	39[e]	58	7[k]	133	3	3
H_1	86	33[e]			130	9	9
H_2	72	18[e]			102	10	12
CH_3CO^+	43	100[e]			100	5	6
					95	29	32
					87	18	22
					82	5	6
					73	42	48
CH_3CO^+	43	100[e]			43	100	100

[a] T_d 190°C; [b] T_d 170°C; [c] T_d 180°C; [d] T_d 180°C; [e] confirmed by high-resolution measurement; [f] $C_8H_{14}NO_3 + C_8H_{12}O_4$; [g] $C_4H_8O_3$; [h] $C_5H_9O_2$; [i] $C_4H_7O_2$; [j] C_4H_7O; [k] C_3H_6O.

(at time 0, 10, 20, and 30 h). After 40 h at 35°C was the reaction stopped and the mixture filtered. Solvent was evaporated and the residue was chromatographed on a Sephadex LH-20 column in methanol. The first fraction was purified by preparative chromatography on Kieselgel F60 in the system S1. Compound *VIII* was obtained (115 mg, 52%), m.p. 245°C (hexane–chloroform),

TABLE IV
Mass spectra of selected compounds

	m/z	*XVI*[a] %	*XX*[b] %	*XXI*[c] %	*XXIV*[d] %
M^+	667	0·01[e]	0·2	0·07	—
M − 18	649	—	0·05	—	—
M − 43	624	0·005[e]	0·02	—	—
Aglycone fragmentation[30]					
	442	—	0·7	0·6	—
	382	7	3	3	—
	362	79[e]	78	82	5
	344	50	32	39	3
	339	23	—	—	—
	337	42	—	—	—
	329	11	7	6	—
	321	13	—	—	—
	319	13	10	12	—
	309	14	3	—	—
	301	29	17	24	1
Sugar fragmentation[31]					
C_1	226	20	18	11	6
D_1	213	6	—	—	—
A_3	192	24	16	10	5
F_1	168	32	14	14	8
D_2	155	35	32	37	30
H_1	140	41	17	36	11
C_2	113	64	59	39	100
C_3	95	20	14	12	10
	89	70	80	68	15
D_2'	86	38	39	47	20
E_2	37	—	—	—	—
$[CF_3]^+$	69	37	24	25	41
E_3	58	64	34	49	23

[a] T_d 190°C; [b] T_d 190°C; [c] T_d 190°C; [d] T_d 150°C; [e] confirmed by high-resolution measurement.

$[\alpha]_D^{20}$ +169 (*c* 0·15, chloroform), R_F 0·45 (S1). For $C_{33}H_{37}NO_{13}$ (655·7) was calculated: 60·45% C, 5·69% H, 2·14% N; found: 60·31% C, 5·42% H, 2·02% N.

Reaction of *IV* with 3-acetamido-4-O-acetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride carried out in tetrahydrofuran gave another product in 69% yield, m.p. 119–122°C. According to mass and NMR spectra (Tables I–III), it was identified as a mixture of two compounds having the structures *X* and *XI*.

(7*S*)-O-(2′-O-(3″-acetamido-2″,3″,6″-trideoxy-α-L-lyxopyranosyloxy)-ethyl)daunomycinone (*IX*)

Solution of compound *VIII* (50 mg, 0·076 mmol) in 0·1M-NaOH (20 ml) was allowed to stand for 1 h and then was carbon dioxide bubbled through. The solution was extracted with chloroform, solvent was evaporated and the residue was chromatographed on Silufol in system S1. Compound *IX* (44 mg, 94%) was obtained; m.p. 223–225°C (chloroform–hexane), $[\alpha]_D^{20}$ +133° (*c* 0·1, chloroform), R_F 0·15 (S1). For $C_{31}H_{35}NO_{12}$ (613·6) was calculated: 60·68% C, 5·75% H, 2·28% N; found: 60·42% C, 5·71% H, 2·19% N.

(7*S*)-O-(2′-O-(3″,4″-di-O-acetyl-2″,6″-dideoxy-α- and β-L-arabinopyranosyloxy)ethyl)daunomycinone (*XII* and *XIII*)

Crude 3,4-di-O-acetyl-2,6-dideoxy-α,β-L-arabinopyranosyl chloride (*II*) (250 mg, 1 mmol), mercuric cyanide (120 mg), mercuric bromide (180 mg), and Potasit 3 Å (3 g) were added to the solution of aglycone *IV* (200 mg, 0·45 mmol) in chloroform (40 ml). The mixture was stirred 15 h at 70°C. Insoluble portions were filtered off, solvent was removed and the residue was chromatographed on a silica gel column in the system S2. Two main fractions were further purified by preparative thin-layer chromatography on Silufol in the system S3. The α-anomer *XII* (85 mg, 28·7%), m.p. 170–172°C (methanol), $[\alpha]_D^{20}$ +146° (*c* 0·3, chloroform), R_F 0·70 (S1) and the β-anomer *XIII* (73 mg, 25%), m.p. 103°C (precipitated with hexane from chloroform solution), $[\alpha]_D^{20}$ +246° (*c* 0·2, chloroform), R_F 0·78 (S1). For $C_{33}H_{36}O_{14}$ (656·4) was calculated: 60·36% C, 5·53% H; found in *XII*: 60·45% C, 5·38% H; found in *XIII*: 60·49% C, 5·40% H.

(7*S*)-O-(2′-O-(2″,6″-dideoxy-α-L-arabinopyranosyloxy)ethyl)daunomycinone (*XIV*)

Excess of 0·5 mmol l^{-1} sodium methoxide was added to the solution of compound *XII* (35 mg, 0·053 mmol) in methanol (15 ml) and the mixture was allowed to stand for 1 h at room temperature. Water (60 ml) was then added, the solution was saturated with gaseous carbon dioxide and extracted with chloroform. Compound *XIV* (29·5 mg, 96%), m.p. 121°C (methanol), $[\alpha]_D^{20}$ +117° (*c* 0·4, chloroform), R_F 0·21 (S1) was isolated from the dried extract upon evaporation of the solvent. For $C_{29}H_{32}O_{12}$ (572·6) was calculated: 60·84% C, 5·63% H; found: 60·57% C, 5·41% H. Similarly was from *XIII* (25 mg, 0·038 mmol) prepared compound *XV* (20 mg, 92%), m.p. 127–128°C (methanol), $[\alpha]_D^{20}$ +95° (*c* 0·1, chloroform), R_F 0·27 (S1). For $C_{29}H_{32}O_{12}$ (572·6) was calculated: 60·84% C, 5·63% H; found: 60·49% C, 5·58% H.

(7*S*)-O-(2′-O-(3″-trifluoroacetamido-2″,3″,6″-trideoxy-α- and β-L-lyxopyranosyloxy)ethyl)daunomycinone (*XVI* and *XVII*)

Mercuric oxide (50 mg), mercuric bromide (70 mg), mercuric cyanide (75 mg), and Potasit 3 Å (4 g) were added to the solution of aglycone *IV* (180 mg, 0·41 mmol) in chloroform (35 ml). 3-Trifluoroacetamido-4-O-trifluoroacetyl-2,3,6-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride (*VII*) was stepwise added (4 × 50 mg, at time 0, 3, 10, and 15 h). The mixture was heated to 50–60°C

for 22 h. Unsoluble portions were filtered off and the solvent was removed. (30 ml) was added and after 1 h reflux was added water and extracted with chloroform. Solvent was removed and the residue was chromatographed on a Sephadex LH-20 column in chloroform. Three fractions were obtained: (i) compound *XVI* (137 mg, 51%) m.p. 247—248°C (methanol), $[\alpha]_D^{20}$ −170·0° (*c* 0·1, chloroform), R_F 0·42 (S1); (ii) compound *XVII* (65 mg, 24%), m.p. 223—225°C (methanol), $[\alpha]_D^{20}$ +166·7° (*c* 0·1, chloroform), R_F 0·48 (S1); (iii) compound *IV* starting (30 mg, 16%). For $C_{31}H_{32}NO_{12}F_3$ (667·3) was calculated: 55·77% C, 4·83% H, 2·10% N; found in *XVI*: 55·62% C, 4·72% H, 2·02% N; found in *XVII*: 55·54% C, 4·69% H, 2·04% N.

(7*S*)-O-(2′-O-(3″-amino-2″,3″,6″-trideoxy-α-L-lyxopyranosyloxy)ethyl)daunomycinone (*XVIII*)

Aqueous 0·1M-NaOH (25 ml) was added to the solution of compound *XVI* (40 mg, 0·06 mmol) in the mixture of methanol (30 ml) and acetone (5 ml). The mixture was left standing 3 h at 28°C. Carbon dioxide was bubbled through and extraction with chloroform was performed. Solvent was removed, an excess of 0·5% oxalic acid was added to the residue and upon neutralization with sodium hydrogen carbonate was the extraction with chloroform repeated. The volume of extract was reduced and the residue was precipitated with hexane. Compound *XVIII* (22 mg, 64%), m.p., 197—198°C, $[\alpha]_D^{20}$ +344° (*c* 0·03, chloroform), R_F 0·27 (S6) was obtained. For $C_{29}H_{33}NO_{11}$ (571·6) was calculated: 60·94% C, 5·82% H, % N; found: 60·85% C, 5·87% H, 2·37% N.

(7*S*)-O-(2′-O-(3″-amino-2″,3″,6″-trideoxy-β-L-lyxopyranosyloxy)ethyl)daunomycinone (*XIX*)

Solution of compound *XVIII* (30 mg, 0·045 mmol) in aqueous 0·1M-NaOH (20 ml) was allowed to stand 1·5 h at 28°C. Carbon dioxide was then introduced and the mixture was extracted with chloroform. Compound *XIX* was prepared in 89·5% yield, m.p. 178°C, $[\alpha]_D^{20}$ +244 (*c* 0·05, chloroform), R_F 0·30 (S6).

(7*R*)-O-(2′-O-(3″-trifluoroacetamido-2″,3″,6″-trideoxy-α-
and β-L-lyxopyranosyloxy)ethyl)daunomycinone (*XX* and *XXI*)

Mercuric oxide (150 mg), mercuric bromide (80 mg), mercuric cyanide (140 mg), and Potasit (3 g) were added to the solution of aglycone *V* (120 mg, 0·27 mmol) in chloroform (30 ml) and nitromethane (5 ml). Solution of *VII* (185 mg, 0·47 mmol) in the 1 : 1 mixture of chloroform and nitromethane (12 ml) was dropwise added. The mixture was stirred 5 h at 60°C. Unsoluble portions were filtered off and solvents were removed. Absolute methanol (40 ml) was added to the residue and refluxed for 1·5 h. Upon evaporation to dryness, water was added and extracted with chloroform. Followed chromatography on Sephadex LH 20 and column chromatography on silica gel in the system S4. The first fraction contained compound *XXI* (15 mg, 8%), m.p. 137°C (chloroform), R_F 0·23 (S1), second fraction — compound *XX* (32 mg, 73%), m.p. 124 to 125°C (chloroform–methanol), R_F 0·32 (S1), third fraction — aglycone *V* (12 mg, 10%).

(7*R*)-O-(2′-O-(3″-amino-2″,3″,6″-trideoxy-α-L-lyxopyranosyloxy)ethyl)daunomycinone (*XXII*)

Solution of compound *XX* (50 mg, 0·075 mmol) in aqueous 0·1M-NaOH (30 ml) was allowed to stand 0·5 h at 28°C, then saturated with carbon dioxide and extracted with chloroform. Free glycoside *XXII*, m.p. 187—188°C (methanol, chloroform–hexane) was isolated in 91% yield. For $C_{29}H_{33}NO_{11}$ (571·6) was calculated: 60·94% C, 5·82% H, 2·45% N; found: 60·71% C, 5·64% H, 2·41% N. Similarly was prepared the glycoside *XXIII* from *XXI* in amount sufficient for biological tests only.

(7*S*)-O-(4'-O-(3"-trifluoroacetamido-2",3",6"-trideoxy-α-L-lyxopyranosyloxy)-2-butynyl)-daunomycinone (*XXIV*)

Mercuric cyanide (180 mg) and Potasit (3 g) were added to the solution of aglycone *VI* (215 mg, 0·46 mmol) in chloroform (35 ml) and nitromethane (10 ml). Portionwise was added chloroform solution of 3-trifluoroacetamido-4-O-trifluoroacetyl-2,3,6-trideoxy-α,β-L-lyxopyranosyl chloride *VII* (370 mg, 148 mmol). The mixture was stirred 6 h at 50°C. Chromatographic separation was performed on a silica gel column in system S5 and on Sephadex LH-20 in chloroform. Three fractions were obtained: (i) compound *XXIV*: (243 mg, 76%), m.p. 193°C (methanol), R_F 0·65 (S1), (ii) unreacted *VI* (37 mg, 17%) (iii) unidentified compound (1·2 mg). For $C_{33}H_{32}NO_{12}F_3$ (691·6) was calculated: 57·31% C, 4·67% H, 2·02% N; found: 57·45% C, 4·83% H, 1·89% N.

(7*S*)-O-(4'-O-(3"-amino-2",3",6"-trideoxy-α-L-lyxopyranosyloxy)-2-butynyl)-daunomycinone (*XXV*)

Deacetylation of *XXIV* in 0·1M-NaOH (2 h, 25°C) provided glycoside *XXV*, m.p. 197°C (chloroform–methanol), R_F 0·44 (S6) in 82% yield. For $C_{31}H_{33}NO_{11}$ (595·6) was calculated: 62·52% C, 5·58% H, 2·35% N; found: 62·27% C, 5·51% H, 2·17% N.

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