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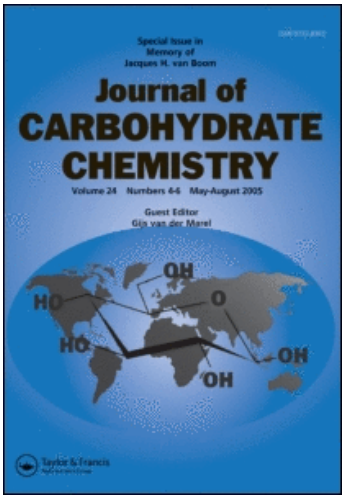

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SYNTHESIS OF THE OLIGOSACCHARIDE MOIETIES OF MUSETTAMYCIN, MARCELLOMYCIN AND ACLACINOMYCIN A, ANTITUMOR ANTIBIOTICS.

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ABSTRACT

Condensation of benzyl 2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (5) with 4-O-acetyl-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl bromide (10) carried out under Koenigs-Knorr conditions gave 12. Total deprotection of 12 and N-dimethylation at C-3 led to 17 while selective removal of the 4-O-acetyl group led to 13, a synthetic intermediate for preparing 24 and 33. Condensation of 13 with di-O-acetyl-L-fucal (18) or 4-O-acetyl-L-amicetal (25) in the presence of N-iodosuccinimide followed by hydrogenolysis of the C-2"-I bond gave 20 and 27 respectively. The trisaccharide 24 then was obtained from 20 by the same sequence of reactions used to convert 12 into 17. After deacetylation and oxidation, this set of reactions also transformed 27 into 33.

INTRODUCTION

During the last decade, several anthracycline antibiotics which exist in the form of oligosaccharide glycosides have been discovered. Among them, musettamycin (1) and marcellomycin (2) were obtained from a fermentation broth of an Actinosporangium species[1] while aclacinomycin A (3) was isolated from Streptomyces galileus MA 144-M1.[2] Structural analyses have shown that 1 is a disaccharide[1] and 2[1] and 3 are trisaccharide derivatives. These two last molecules are highly active and, more importantly, exhibit much lower cardiotoxicity[4] than the doxorubicin and daunorubicin used today in cancer chemotherapy.[5]

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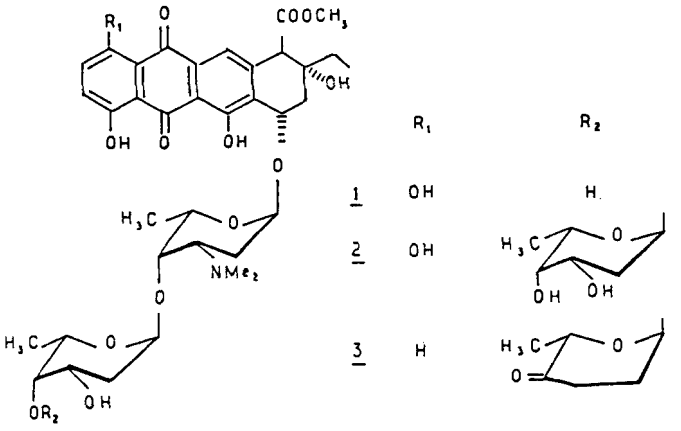
Starting from the aglycon (ε-pyrromycinone, in the case of 1 and 2, and aklavinone, in the case of 3), the sugar sequences of the



oligosaccharide moieties are as follows: L-rhodosamine or (N,N-dimethyl-L-daunosamine) (unit A), 2-deoxy-L-fucose (unit B) and a second 2-deoxy-L-fucose (unit C in 2) or a L-cinerulose (unit C in 3), all with α(1-4)glycosidic interlinkages. The widespread occurrence of these oligosaccharides is underscored by the fact that they have also been found in other anthracyclines such as the disaccharide of 1 in collinemycin,[6,7] auramycin C,[8] sulfurmycin C,[8] the trisaccharide of 2 in mimimycin[6,7] and the trisaccharide of 3 in auramycin A,[9] sulfurmycin A,[9] trisarubicinol[10] and betaclamycin A.[8]

Syntheses of methyl glycosides of the disaccharide unit A-B[11] or B-C[12] or syntheses of methyl glycosides closely related to them[13] have been previously reported but none of them were suitable for complete elaboration of the di- or trisaccharide skeleton of 1, 2 and 3.

A logical approach to the synthesis of such oligosaccharides would be through the glycosidation of benzyl hexopyranoside as precursor of unit A with a suitably protected 2-deoxy-L-fucose derivative (unit B). Selective deprotection of O-4' of the disaccharide unit A-B followed by glycosidation under neutral conditions with a glycal precursor of unit C would then provide the trisaccha-

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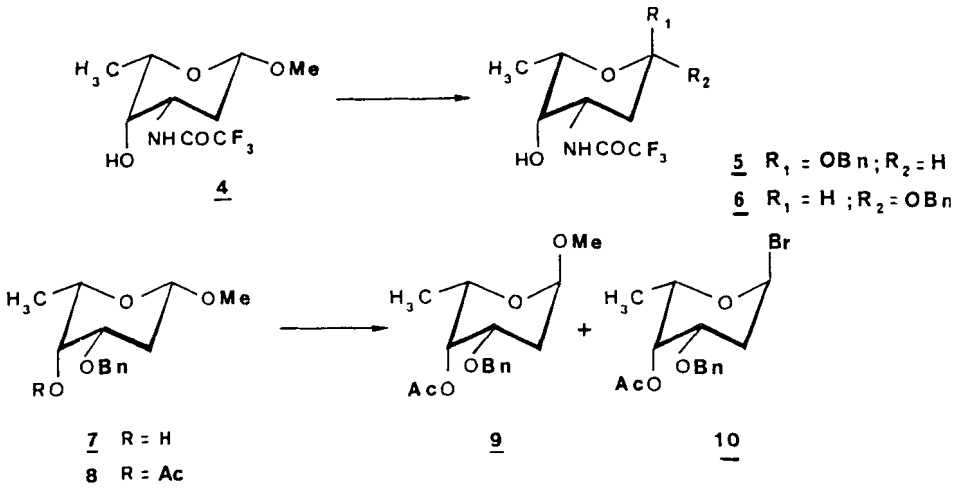
ride skeletons. After suitable transformations, final hydrogenolysis could be used to remove the benzyl group at C-1 without effecting the interglycosidic bonds.[14]

RESULTS AND DISCUSSION

The monosaccharides used as the respective precursors of these different units were benzyl N-(trifluoroacetyl)-α-L-daunosaminide (5), 4-O-acetyl-3-O-benzyl-2-deoxy-α-L-fucosyl bromide (10) and 3,4-di-O-acetyl-L-rhamnal (18) or 4-O-acetyl-L-amicetal (25).

L-Daunosamine derivative 5 was synthesized in one-step and in 89% yield from methyl N-trifluoroacetyl-β-L-daunosaminide 4[15] via a stereoselective transglycosylation reaction. In addition, acetylation of methyl 3-O-benzyl-2,6-dideoxy-β-L-lyxo-hexopyranoside 7 which was obtained in 90% yield by regioselective alkylation of the corresponding diol via its stannylene acetal,[12b,16] led to 8 in almost quantitative yield. Further treatment of 8 with bromotrimethylsilane[17] in benzene at room temperature gave the bromo derivative 10 along with methyl 3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranoside (9) and a small amount of unreacted starting material 8. As the instability of 10 did not permit purification, the crude reaction mixture was used in the subsequent coupling reaction of 5 with 10. The methyl glycoside 9 could be easily separated after the glycosidation-step and reacted with bromotrimethylsilane to afford additional 10.



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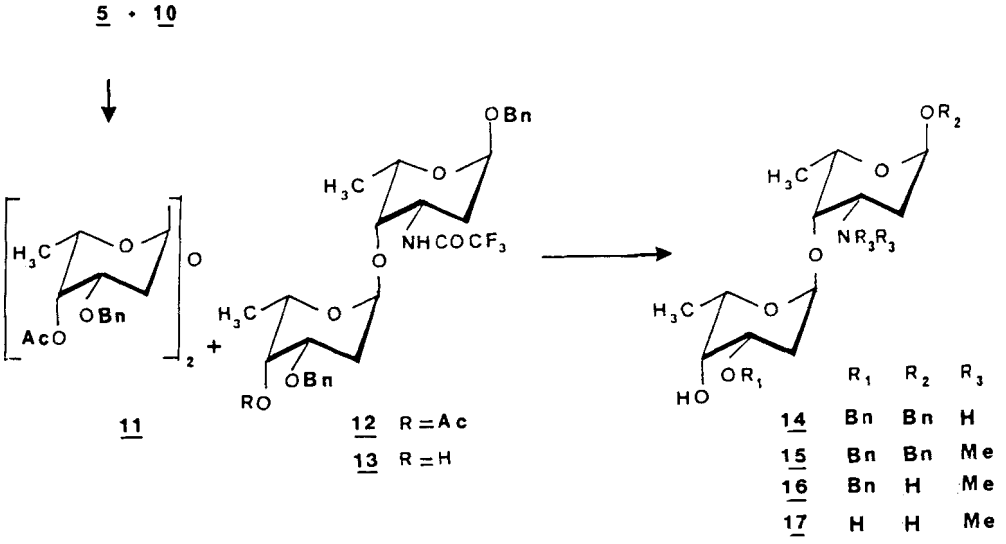
Synthesis of the Disaccharide Moiety of Musettamycin.

Glycosidation of 5 with the crude bromo derivative 10 under Koenigs-Knorr conditions led to a mixture of the desired disaccharide 12 (40%) along with a dimeric compound 11[18] and unreacted 9. After chromatographic separation, transesterification of 12 gave 13 in quantitative yield and alkaline treatment of 12 or 13 afforded the amino derivative 14 (95%). This later compound was immediately N-methylated by treatment with formaldehyde in the presence of $NaBH_3CN$ yielding 15 in 85% yield.

Treatment of 15 under various conditions (H_2 and Pd-$BaSO_4$ or Pd-C in EtOH-AcOH solution or Pd-C in EtOH-HCOOH solution) resulted in either the recovery of the starting material or in the formation of many side-products. The partial conversion of 15 into the free disaccharide moiety of musettamycin was finally conducted under H_2 (1 atm) with Pd-on-charcoal as catalyst in methanolic 0.2N HCl solution for a short time (30 min) but instead of obtaining 16, the partially deprotected disaccharide 17 was isolated in low yield along with unreacted 15. Compound 17 was characterized by ^1H NMR spectroscopy and by mass spectrometry.[19] The mass spectrum of 17 exhibited a base peak (DCI/NH_3) at m/z 266 characteristic of the ion related to the benzyl glycoside of a rhodosaminyl residue. There also was an intense ion at m/z 396 corresponding to $|M+H^+|$. Easier hydrolysis of the C-3' benzyl ether when compared to the anomeric acetal can be explained by stereoelectronic effects of the later especially in acidic medium where 15 probably exists as its ammonium salt.



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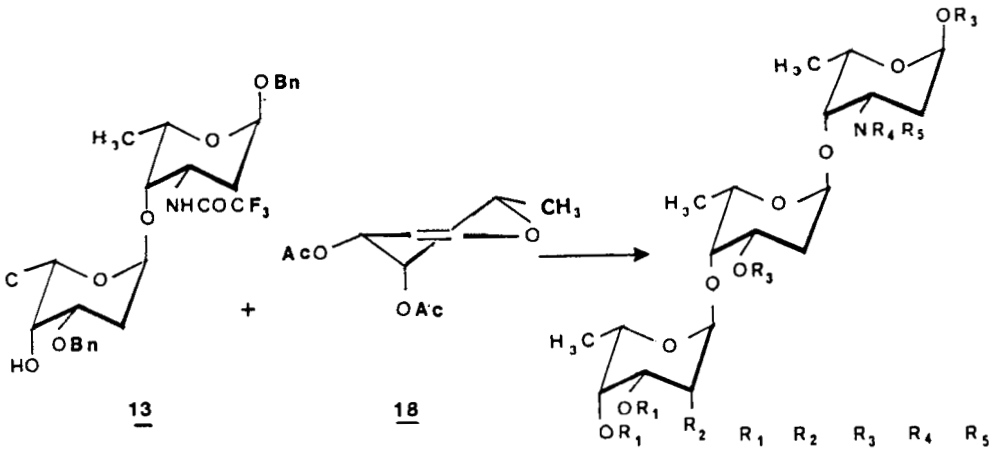
Synthesis of the Trisaccharide Moiety of Marcellomycin

Disaccharide 13 was used as the starting material in further experiments to synthesize the trisaccharide moieties of marcellomycin and aclacinomycin A. In the former case, coupling of 13 with an excess of 3,4-di-O-acetyl-L-fucal (18) (2 molar equivalents) was performed in the presence of N-iodosuccinimide[20] affording stereoselectively (70%



	R1	R2	R3	R4	R5
19	Ac	I	Bn	H	$COCF_3$
20	Ac	H	Bn	H	$COCF_3$
21	H	H	Bn	H	H
22	H	H	Bn	Me	Me
23	Ac	H	H	H	$COCF_3$
24	H	H	H	Me	Me

yield) the trisaccharide 19 as a crystalline compound. The α linkage between the units B and C was unambiguously established by 270 MHz ^{1}H NMR spectroscopy with a characteristic signal for 1"-H at δ 5.10 ppm with a small coupling constant ($J_{1",2"} < 1$ Hz). Hydrogenolysis of the $C_{2"}$-I bond of 19 leading to 20 (80% yield) was followed by deacetylation under alkaline conditions to give the amino derivative 21 as a syrup. Dimethylation of 21 as described above during the preparation of 16 provided compound 22.

While the trisaccharide 20 was easily hydrogenolyzed to give 23 in 60% yield as a mixture of anomers, difficulties in removing the benzyl groups were encountered with 22 as with 15 (vide infra), proba-

bly due to the basic dimethylamino group. As with the formation of 17, the conversion of 22 into the free trisaccharide moiety of marcellomycin 24 was carried out under hydrogen atmosphere with Pd-on-charcoal under slightly acidic conditions.

Synthesis of the Trisaccharide Moiety of Aclacinomycin A.

In a similar manner to the synthesis of the trisaccharide moiety of marcellomycin, glycosidation of the disaccharide 13 was carried out in the presence of N-iodosuccinimide[20] but with 4-O-acetyl-L-amicetal (25) (4 mol equiv) instead of per-O-acetyl-L-rhamnal. The trisaccharide 26 was obtained stereospecifically in 68% yield after chromatography. The α linkage between the (B) and (C) units was established by a characteristic signal for 1"-H at δ 5.07 (broad singlet) in the 400 MHz ^1H NMR spectrum. Hydrogenolysis of the C-2"-I bond of 26 was followed by transesterification of 27 to provide 28 quantitatively. Oxidation of 28 with pyridinium dichromate afforded 29 (90%) which was transformed into the amino derivative 30 and methylated with formaldehyde in the presence of $NaBH_3CN$ in acetonitrile to give 31 (90% yield).

Deprotection of the trisaccharide 29 by hydrogenolysis in ethyl acetate yielded the free trisaccharide 32 in 85% yield after chromatography. The corresponding free N,N-dimethyl trisaccharide could not be obtained in a satisfactory yield and purity.

Although the general scheme which is reported in this paper was convenient to synthesize the sugar moieties of anthracycline class II, musettamycin, marcellomycin and aclacinomycin, use of a benzyl ether as an anomeric protecting group was not quite satisfactory for obtaining free oligosaccharides with an N,N'-dimethylamino group. However coupling of these di- or trisaccharides with different aglycons can be achieved with the N-trifluoro acetyl derivatives such as 23 or 32, and the dimethylamino group at C-3 can be introduced later on.

EXPERIMENTAL

General Methods and Material. Melting points were determined on a Hofler hot-stage microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer, calibrated against polystyrene film and are expressed in cm^{-1}. ^1H NMR spectra

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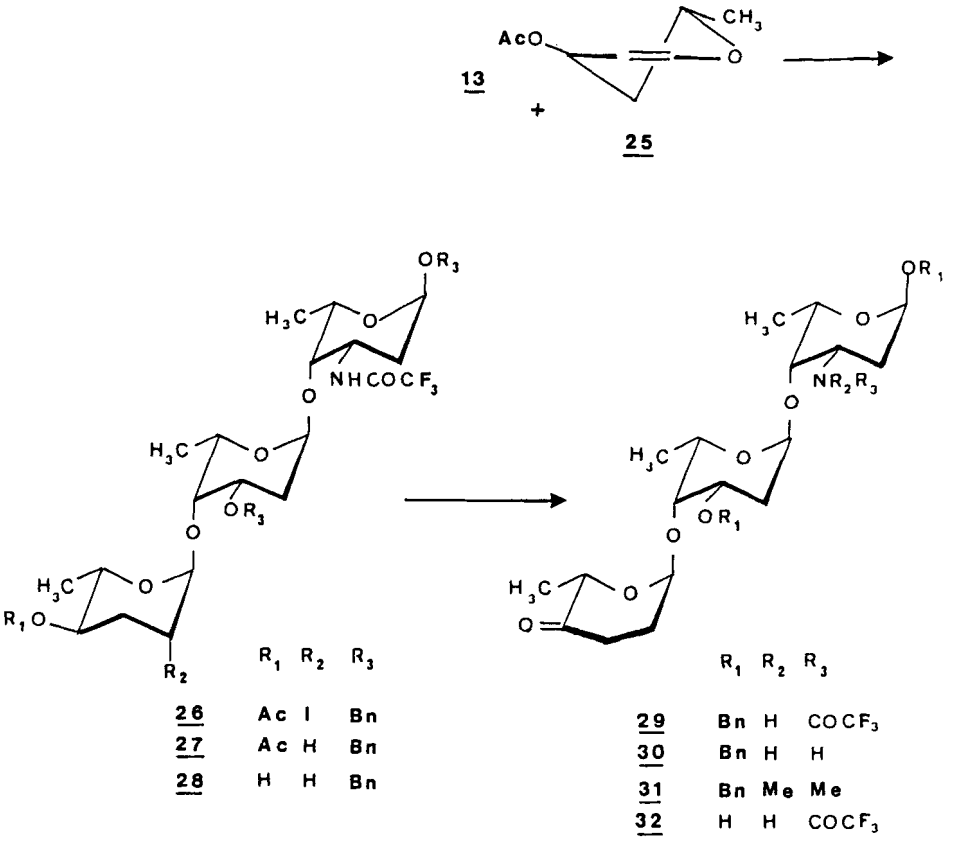

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were obtained on a Bruker HX 270 in $CDCl_3$ (s, singlet; d, doublet; m, multiplet; q, quadruplet) (Chemical shifts are relative to TMS (δ= 0.0) and coupling constants are in Hertz). Mass spectra (DCI/NH_3) were recorded on a Nermag R 1010C. Silica gel for column chromatography or flash chromatography was Merck silica gel H.60 n°7736. Microanalyses were performed by the "Laboratoire de Microanalyse du CNRS" Gif-sur-Yvette.

Benzyl 2,3,6-Trideoxy-3-trifluoroacetamido-α-L-lyxo-and β-L-lyxo-hexopyranoside, (5 and 6). To a solution of methyl 2,3,6-trideoxy-3-trifluoroacetamido-β-L-lyxo-hexopyranoside[15] (260 mg, 1 mmol) in benzyl alcohol (2 mL) and hexane (25 mL) was added p-toluenesulfonic acid (170 mg). The mixture was refluxed for 3 h in a Dean-Stark apparatus and neutralized by addition of sodium hydrogen carbonate solid (100 mg).

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Table 1 : ^1H NMR Data of mono and disaccharides derivatives[a]

	5	6	8	9	11	12	13	14	15
H_1	4,97	4.57	4.35	4.86	5.23	4.94	4.93	4.95[b]	5.04[b]
H_{2a}	1.80	1.63	1.87	2.00	2.08	1,76	1.75	1.57-2.33	1,82-2.93
H_{2e}	1.97	2.09	2.01	1.92	1.80	1.90	1.90	1.57-2.33	1.82-2.93
H_3	4.47	4.10	3.58	3.91	3.87	4.55	4.48-4.57		
H_4	3.60	3.52	5.27	5.34	5.34	3.47	3.45	3.47	3.78
H_5	4.08	3.63	3.57	3.95	3.92	4.01	4.01	3.92[c]	3.85[c]
H_6	1.22	1.34	1.25	1.18	1.13	1.17	1.17	1.18	1.20
$H_{1'}$						4.94	4.93	5.00[b]	5.09[b]
$H_{2a'}$						2.10	2.07	1.57-2.33	1.82-2.93
$H_{2e'}$						1.99	1.97	1.57-2.33	1.82-2.93
$H_{3'}$						4.03	3.97	3.97	4.00
$H_{4'}$						5.36	3.82	3.83	3.82
$H_{5'}$						4.17	4.06	4.12[c]	4.44[c]
$H_{6'}$						1.18	1.32	1.28	1.24
CH_2Bn	4.52;4.68	4.63;4.92	.47;4.70	4.43;4.69	4.42;4.72	4.50;4.65 4.42;4.69	4.50;4.65 4.57;4.61	4.48;4.65 4.57;4.61	4.67;4.51 4.45;4.59
NH	6,77	6,86				8,17	8,44		
OMe			3.51	3.33					
OAc			2.17	2.17	2.18	2.16			
NMe_2									2.38

[a] in $CDCl_3$ [b,c] assignments might be interchanged.

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Table 2 - ^{1}H NMR data of trisaccharide derivatives[a]

	19	20	21	22	26	27	28	29	30	31	32 α	32 β
H_1	4.95	5.00	4.88[b]	5.22[b]	4.90	4.95	4.92	4.92	5.02	5.00[b]	5.37	
H_2	1.62-2.23	1.69-2.27	1.53-1.98	2.15-2.58	1.72 1.88	1.75 2.30	1.68-2.01	1.73 1.88	1.58-2.32	1.96-2.74	1.78 1.88	1.92 2.23
H_3			3.12		4.50	4.44	4.48	4.50	3.23			
H_4	3.48	3.50	3.47		3.44	3.47	3.43	3.44	3.44	3.50		
H_5	4.03	4.08	4.07		3.98	4.01	3.98	3.99	3.85[b]			
H_6	1.17	1.18	1.08	1.33[c]	1.15	1.15	1.15	1.16	1.18	1.16	1.22	1.33
$H_{1'}$	4.95	5.00	4.92[b]	5.32[b]	4.90	4.95	4.92	4.92	4.93	5.10		
$H_{2'}$	1.62-2.23	1.69-2.27	1.53-1.98	2.15-2.58	1.93-2.25	1.79-2.30	1.68-2.01	1.98-2.11	1.58-2.32	1.96-2.74	1.92-2.23	
$H_{3'}$	3.96	3.98	3.80		3.92	3.97	3.88[b]	3.96	3.96[b]			
$H_{4'}$	3.90	3.92	3.90		3.88	3.91	3.88	3.99	3.96			
$H_{5'}$	4.07	4.16	4.10		4.04	4.08	4.05	4.07	4.11			
$H_{6'}$	1.24	1.25	1.12	1.38[c]	1.24	1.24	1.25	1.27	1.23	1.25		
$H_{1''}$	5.16	5.10[b]	4.99	5.43	5.06	4.87	4.82	5.06	5.07	5.25[b]	5.09	5.12
$H_{2''}$	4.45	1.69-2.27	1.53-1.98	2.15-2.58	4.43	1.79-2.30	1.68-2.01	2.11-2.60	2.16-2.65	1.96-2.74	1.92-2.23	
$H_{4''}$		5.15[b]			4.90	4.95	3.18					
$H_{5''}$		4.48			4.25	4.18	3.96[b]	4.57			4.49[b]	4.51[b]
$H_{6''}$	0.84	0.77	0.82	1.38[c]	0.95	0.89	1.00	0.97	0.93	0.88		
CH_2Bn	4.52;4.67 4.58;4.63	4.54;4.67 4.60;4.70	4.42;4.54 4.58;4.72	4.58;4.60 4.80;4.97	4.48;4.63 4.55;4.67	4.51;4.59 4.67;4.73	4.48;4.63 4.55;4.70	4.48;4.63 4.54;4.64	4.46;4.63 4.56;4.65	4.52;4.68		
Others :												
NH	8.27	8.41			8.22	8.33	8.29	8.25			8.40	8.59
OAc	2.07 2.18	2.00 2.14			2.01	2.03						
$H_{3''}$		5.38			1.93-2.25							
NMe_2				2.75						2.34		

[a] in $CDCl_3$ [b,c] assignments might be interchanges

After evaporation under reduced pressure, the residue was diluted with dichloromethane and the insoluble material removed by filtration. Evaporation of the solvent under reduced pressure afforded a residue (390 mg) which was chromatographed on silica gel. Elution with a mixture of hexane-ethyl acetate (5:1) gave successively the α-L-anomer 5[13b] (300 mg, 89%), a mixture of α and β anomers, 5 and 6 (68 mg) and a small amount of the β-L-anomer 6 (12 mg).

α-L-anomer, 5: syrup $|\alpha|_D^{20}$ -69° (c 1.75, chloroform); IR (film): 3500, 3300 (OH, NH), 1690 (amide) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{15}H_{18}O_4NF_3$(333.29): C, 54.05; H, 5.43; O, 19.20; Found: C, 54.12; H, 5.50; O, 19.15.

β-L-anomer, 6: mp 117-120 °C (ether); $|\alpha|_D^{20}$ +67° (c 1.2, chloroform); IR (film): cf. 5.

Anal. Calcd for $C_{15}H_{18}O_4NF_3$ (333.29): C, 54.03; H, 5.43; O, 19.18. Found: C, 54.20; H, 5.30; O, 19.18.

Methyl 4-O-Acetyl-3-O-benzyl-2,6-dideoxy-β-L-lyxo-hexopyranoside (8). To a solution of 7[16] (6 g, 23.8 mmol) in anhydrous pyridine (20 mL), acetic anhydride (10 mL) was slowly added. After stirring for 15 h at room temperature, the reaction mixture was extracted with dichloromethane and the organic solution was washed with cold 1N aqueous H_2SO_4 solution, saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated *in vacuo*. This yielded a crude product (6.9 g) which was crystallized from ether giving 8 (6 g, 85%): mp 60 °C; $|\alpha|_D^{20}$ (c 2.2, chloroform); IR (Nujol): 1700 (ester) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{16}H_{22}O_5$ (294.32): C, 65.29; H, 7.49; O, 27.18; Found: C, 65.30; H, 7.35; O, 27.30.

Benzyl 4-O-(4-O-Acetyl-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (12). To a solution of 8 (3.7 g, 12.6 mmol) in anhydrous benzene (40 mL) was added at 0 °C under an argon atmosphere, freshly distilled trimethylsilyl bromide (4 mL). After the mixture was stirred at room temperature for 18 h, the solvent was evaporated under reduced pressure to give a mixture of 8, 9 and 10. To a solution of 5 (1 g, 3 mmol) in dry dichloromethane (50 mL) were added yellow silver oxide (1.5 g), mercuric bromide (130 mg) and powdered 4Å molecular sieves (13 g).

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After stirring for 5 min, the residue containing the crude bromo derivative 10 was dissolved in dry dichloromethane (10 mL) and added, at 0 °C to the reaction mixture. Stirring was maintained at 0 °C for 5 min and then at room temperature for 1 h. After filtration, the filtrate was evaporated in vacuo and the residue (3.4 g) chromatographed on silica gel. Elution with a mixture of hexane-acetone (4:1) afforded successively the unreacted methyl α-L-glycoside 9 (1.5 g) and a mixture of 11 and 12. This mixture was chromatographed once again on silica gel and eluted with a mixture of toluene-ethyl acetate (95:5) to give the disaccharide 12 (710 mg, 40% from 5) and the dimer 11 (800 mg). Crystallization of 12 from hexane-ether (1:1) gave: mp 127-128 °C, $|\alpha|_D^{20}$ -167° (c 1.8, chloroform); IR (Nujol); 3280 (NH), 1750-1700 (ester) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{30}H_{36}NO_8F_3$ (595.62): C, 60.50; H, 6.09; N, 2.35; F, 9.57. Found: C, 60.66; H, 6.15; N, 2.57; F, 9.51.

Methyl glycoside 9: mp 89-91 °C (ether); $|\alpha|_D^{20}$ -167° (c 1.8, chloroform); IR (Nujol): 3280 (NH), 1750-1700 (ester,amide), 1510 cm^{-1} (CH_2Ar).

Anal. Calcd $C_{16}H_{22}O_5$ (294.35): C, 65.29; H, 7.49; O, 27.18. Found: C, 65.50; H, 7.37; O, 27.12.

Dimeric compound 11: mp 120 °C (ether); IR (Nujol): 1740 (ester) and 1510 cm^{-1} (CH_2Ar); MS (DCI/NH_3): m/z 560 (M^++NH_4^+), 280 and 263.

Anal. Calcd for $C_{30}H_{38}O_9$ (542.63): C, 66.40; H, 7.06; O, 26.54. Found: C, 66.45; H, 7.13; O, 26.30.

Benzyl 4-O-(3-O-Benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (13). A solution of 12 (1.57 g, 2.64 mmol) in methanol (50 mL) and 1M methanolic sodium methoxide (5 mL) was stirred at room temperature for 18 h. After neutralization with ion-exchange resin Amberlite IR-50 (H^+), evaporation of the solvent in vacuo afforded 13 (1.41 g, 97%) as a syrup: $|\alpha|_D^{20}$ -123° (c 1.64, chloroform); IR (film): 3520, 3240 (OH, NH), 1730 (amide) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{28}H_{34}O_7NF_3$ (553.58): C, 60.75; H, 6.19; N, 2.53; F, 10.29. Found: C, 59.80; H, 6.25; N, 2.69; F, 10.60.

Benzyl 3-Amino-4-O-(3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2-deoxy-α-L-lyxo-hexopyranoside (14). A solution of 12 (1.1 g, 1.84 mmol) in methanol (60 mL) and water (24 mL) was stirred for 3 h

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at 0 °C in the presence of potassium carbonate (9 g). The solution was neutralized with Amberlite IR-50 (H^+) and evaporated *in vacuo* to give a residue which was purified by flash chromatography. Elution with dichloromethane-methanol (9:1) afforded 420 mg (50%) of 14: syrup, $|\alpha|_D^{20}$ -125° (c 0.4, chloroform); IR (film): 3350 (NH, OH), 1600 and 1510 cm^{-1}.

Anal. Calcd for $C_{26}H_{43}O_6N$ (465.63): C, 67.06; H, 9.30; O, 20.61. Found: C, 67.10; H, 9.33; O, 20.50.

Benzyl 3-*N*,*N*-dimethylamino-4-*O*-(3-*O*-benzyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (15). To a solution of 14 (770 mg, 1.65 mmol) in methanol (20 mL) were added, sodium cyanoborohydride (350 mg, 5.57 mmol), acetic acid (0.2 mL) and 30% formaldehyde (12 mL). After stirring for 5 h at room temperature, extraction with ethyl acetate gave 990 mg of crude oily product. Purification by column chromatography (dichloromethane-methanol, 98:2 then 95:5) led to 650 mg (80%) of 15: $|\alpha|_D^{20}$ -116.5° (c 0.7, chloroform); IR (film): 3560 (OH), 1500 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{28}H_{39}O_6N$ (485.61): C, 69.25; H, 8.09; O, 19.76. Found: C, 69.38; H, 8.12; O, 19.63.

Benzyl 3-*N*,*N*-Dimethylamino-4-*O*-(2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-2,6-dideoxy-α-L-*lyxo*-hexopyranoside (17). A solution of 15 (50 mg) in ethanol (10 mL) was stirred under a hydrogen atmosphere in the presence of palladium on charcoal (50 mg) and 2 drops of concentrated HCl. After stirring for 1 h, neutralization with solid $NaHCO_3$ was followed by filtration and concentration *in vacuo*. A flash chromatography of the residue with dichloromethane-methanol (80:20) gave 5 mg of 17; a syrup, NMR (mixture of α and β anomers): 7.54-7.40 (m, 5H, Ar), 4.95-4.53 (m, CH_2Ar), 2.73 and 2.36 (s, NMe_2), 1.59, 1.57, 1.33 and 1.31 (d, CH_3-6 and 6'); MS (DCI/NH_3): m/z 396 ($M+H^+$), 266 (base peak). Further elution afforded 35 mg of starting material, 15.

Benzyl 4-*O*-(4-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-2-iodo-α-L-*talo*-hexopyranosyl)-3-*O*-benzyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-*lyxo*-hexopyranoside (19). To a solution of 13 (700 mg, 1.26 mmol) and di-*O*-acetyl-L-fucal 18 (560 mg, 2.6 mmol) in acetonitrile, was added *N*-iodosuccinimide (600 mg, 2.66 mmol). After stirring for 1 h at room temperature, di-*O*-acetyl-L-fucal

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18 (560 mg) and NIS (600 mg) were added and the stirring prolonged for 1 h. The crude reaction mixture was evaporated under reduced pressure and the residue (3.2 g) dissolved in dichloromethane. The organic solution was washed with an aqueous solution of sodium thiosulfate, followed by usual work-up. The residue (2.7 g) was chromatographed and elution with hexane-ethyl acetate (6:1) provided 800 mg of 19 (70% from 13) as a crystalline compound: mp 164 °C; $|\alpha|_D^{20}$ -145° (c 1.4, chloroform); IR (Nujol): 3300 (NH), 1750, 1720, 1710 (ester, amide) and 1510 cm^{-1} (CH_2Ar); MS (DCI/NH_3): m/z 911 ($M+NH_4^+$), 785 ($M+NH_4^+-I$) and 351.

Anal. Calcd for $C_{38}H_{47}NO_{12}F_3I$ (893.69): C, 51.07; H, 5.30; I, 14.20. Found: C, 51.20; H, 5.25; I, 14.10.

Benzyl-4-O-(4-O-(3,4-Di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoro-acetamido-α-L-lyxo-hexopyranoside (20). A solution of 19 (550 mg, 0.6 mmol in 10 mL of ethanol) was stirred for 2 h under hydrogen atmosphere (1 atm) in the presence of 10% palladium on charcoal (100 mg) and triethylamine (0.5 mL). The catalyst was removed by filtration and the filtrate concentrated in vacuo. The residue was dissolved in dichloromethane. Usual work-up gave 380 mg (81%) of 20: $|\alpha|_D^{20}$ -159° (c 0.54, chloroform); IR (film); 3260 (NH), 1760-1710 (ester, amide) and 1500 cm^{-1} (CH_2Ar); MS (DCI/NH_3): m/z 785 ($M+NH_4^+$, base peak).

Anal. Calcd for $C_{38}H_{48}NO_{12}F_3$ (767.79): C, 59.44; H, 6.30; O, 25.00. Found: C, 59.55; H, 6.27; O, 25.10.

Benzyl-3-Amino-4-O-(3-O-benzyl-4-O-(2,6-dideoxy-α-L-lyxo-hexopyranosyl)-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-α-L-lyxo-hexopyranoside (21). To a solution of 20 (125 mg, 0.16 mmol, in 5 mL of methanol) was added an aqueous solution of potassium carbonate (400 mg in 2 mL). After stirring for 18 h at room temperature, evaporation in vacuo followed by chromatography with dichloromethane-methanol (7:1) as eluant gave 21 (67 mg, 71%): $|\alpha|_D^{20}$ -164° (c 1, MeOH); IR (film): 3600-3300 (OH, NH), 1500 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{32}H_{45}NO_9$ (587.70): C, 65.39; H, 7.72; O, 24.50. Found: C, 65.50; H, 7.69; O, 24.48.

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Benzyl 4-O-(3-O-Benzyl-4-O-(2,6-dideoxy-α-L-lyxo-hexopyranosyl)-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-N,N-dimethylamino-α-L-lyxo-hexopyranoside (22). Aqueous formaldehyde (37%, 0.25 mL) and sodium cyanoborohydride (62 mg, 1 mmol) were added to a solution of 21 (50 mg, 0.085 mmol) in acetonitrile (5 mL) stirred at room temperature. Evaporation of the solvent in vacuo followed by chromatography with dichloromethane-MeOH (9:1) as eluant afforded 22 (45 mg, 86%); $|\alpha|_D^{20}$ -234° (c 1, chloroform); IR (film): 3580-3400 (OH) and 1500 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{34}H_{49}NO_9$ (615.75); C, 66.32; H, 8.02; O, 22.38. Found: C, 66.38; H, 8.10; O, 22.27.

4-O-(4-O-(3,4-Di-O-acetyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranose (23). A solution of 20 (150 mg, 0.2 mmol) in ethylacetate (10 mL) was vigorously stirred for 30 min under hydrogen atmosphere (1 atm) in the presence of 10% palladium on charcoal. The catalyst was filtered off and the solvent evaporated under reduced pressure to afford 90 mg (60%) of 23 as a mixture of α and β anomers which were not separated, ^1H NMR for the mixture, δ 8.52 and 8.30 (d, NH), 5.32-5.28 (m, H-3", H-4"), 5.19, 5.01, 4.88 (3 broad s, H--1e, H-1', H-1"), 4.87 (dd, H-1a), 4.46 (m, H-5), 4.26-4.10 (m, H-5, H-5', H-5", H-4, H-4'), 3.65 and 3.45 (broad s, H-3, H-3'), 2.15 (s, 2 OAc), 1.23 (d, CH_3-6'), 1.14 (d, CH_3-6), 0.88 (d, CH_3-6"); MS (DCI/NH_3); m/z 605 (base peak $M+NH_4^+$), 587 (M^+), 380, 362, 215, 155, 95.

4-O-(4-O-(2,6-Dideoxy-α-L-lyxo-hexopyranosyl)-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-N,N-dimethylamino-L-hexopyranoses (24). The procedure for preparation of 17 was used. Thus, 22 (45 mg) afforded 24 (22 mg, 70%) as a mixture of diastereoisomers which were not separated. ^1H NMR for the mixture showed complete disappearance of aromatic protons but was too complex for proton assignments: MS (DCI/NH_3): m/z 436 ($M+H^+$), 306, 288, 278, 176, 148, 130, 113.

Benzyl 4-O-(4-O-(4-O-Acetyl-2,3,6-trideoxy-2-iodo-α-L-arabino-hexopyranosyl)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranozyl)-2,3,6-trideoxy-α-L-lyxo-hexopyranoside (26). To a cold solution (0-5 °C) of 13 (1.41 g, 2.55 mmol) in acetonitrile (80 mL) were successively added

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the amicetal derivative 25 (800 mg, 5.12 mmol) and N-iodosuccinimide (1.15 g, 5.12 mmol). Equal amounts of 25 and NIS were added after 2 h and stirring was continued at 0 °C for 1 h. The same work-up as used previously (cf. preparation of 19) gave a residue (4.2 g) which was chromatographed with toluene-acetone (19:1) as eluant. Compound 26 (1.45 g, 68%) was isolated as a syrup: $|\alpha|_D^{20}$ -148° (c 1.7, chloroform); IR (film): 3280 (NH), 1770-1720 (ester, amide) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{36}H_{45}O_{10}NF_3I$ (835.66): C, 51.74; H, 5.42; I, 15.18. Found: C, 51.88; H, 5.50; I, 15.02.

Benzyl 4-O-(4-O-(4-O-Acetyl-2,3,6-trideoxy-α-L-erythro-hexopyranosyl)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (27). Treatment of 26 (700 mg) under the conditions previously used for the preparation of 20 gave 27 (520 mg, 87%); $|\alpha|_D^{20}$ -138° (c 1, chloroform); IR (film): 3280 (NH), 1770-1720 (ester, amide), 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{36}H_{46}NO_{10}F_3$ (709.76): C, 60.92; H, 6.53; O, 22.54. Found: C, 61.05; H, 6.48; O, 22.67.

Benzyl 4-O-(4-O-(2,3,6-Trideoxy-α-L-erythro-hexopyranosyl)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (28). To a stirred solution of 27 (940 mg, 1.32 mmol) in methanol (20 mL) was added a 1 M solution of sodium methoxide in methanol (2 mL). After 1 h the reaction mixture was neutralized with Amberlite IR-50 (H^+) resin. Evaporation of the filtrate afforded 28 (840 mg, 95%) as a syrup, $|\alpha|_D^{20}$ -171° (c 1, chloroform); IR (film): 3450 and 3280 (OH, NH), 1740 (amide) and 1510 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{34}H_{44}NO_9F_3$ (667.73): C, 61.15; H, 6.64; O, 21.56. Found: C, 61.30; H, 6.58; O, 21.47.

Benzyl 4-O-(4-O-(2,3,6-Trideoxy-α-L-glycero-hexopyranosid-4-ulose)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranoside (29). To a solution of 28 (111 mg, 0.16 mmol) in dichloromethane, free of ethanol (20 mL) were added powdered 3 Å Molecular sieves (650 mg) and pyridinium dichromate (300 mg, 0.8 mmol). After stirring for 1 h at room temperature, the reaction

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mixture was diluted with ether and filtered over celite. Evaporation of the filtrate under reduced pressure afforded 29 (100 mg, 91%) which crystallized from hexane-ether; mp 115-116 °C, $|\alpha|_D^{20}$ -213° (c 1.62, chloroform); IR (Nujol): 3280 (NH), 1760-1710 (amide) and 1510 cm^{-1} (CH_2Ar); SM (DCI/NH_3): m/z 683 ($M+NH_4^+$).

Anal. Calcd for $C_{34}H_{42}NO_9F_3$ (665.71); C, 61.34; H, 6.36; O, 21.63. Found: C, 61.50; H, 6.40; O, 21.50.

Benzyl-4-O-(4-O-(2,3,6-Trideoxy-α-L-glycero-hexopyranosid-4-ulose)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside (30). In a manner similar to that described for the preparation of 21, 29 (200 mg) afforded 116 mg (68%) of pure 30 after chromatography of the crude residue (180 mg) using dichloromethane-methanol (9:1). Compound 30 was a syrup, $|\alpha|_D^{20}$ -203 (c 1.3, chloroform); IR (film): 3400, 3280 (NH), 1725 (C=O) and 1500 cm^{-1} (CH_2Ar).

Anal. Calcd for $C_{32}H_{43}NO_8$ (569.67): C, 67.45; H, 7.63; O, 22.46. Found: C, 67.60; H, 7.70; O, 22.28.

Benzyl-4-O(4-O-(2,3,6-Trideoxy-α-L-glycero-hexopyranosid-4-ulose)-3-O-benzyl-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-dimethylamino-α-L-lyxo-hexopyranoside (31). The procedure for preparation of 22 was used. Thus, 30 (26 mg) gave 31 (24 mg, 90%) as a syrup, $|\alpha|_D^{20}$ -172° (c 1.14, chloroform); IR (film): 1725 (C=O) and 1500 cm^{-1} (CH_2Ar); SM (DCI/NH_3): m/z 598 ($M+H^+$), 486, 350, 266 (base peak).

Anal. Calcd for $C_{34}H_{47}O_8N$ (597.74): C, 68.31; H; 7.92; N, 2.34. Found: C, 68.35; H, 7.87; N, 2.25.

4-O-(4-O-(2,3,6-Trideoxy-α-L-glycero-hexopyranosid-4-ulose)-2,6-dideoxy-α-L-lyxo-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetamido-α-L-lyxo-hexopyranose (32). A solution of 29 (100 mg) in ethyl acetate (5 mL) was stirred for 30 min under hydrogen atmosphere (1 atm) in the presence of palladium-on-charcoal (100 mg). After the catalyst was removed by filtration, concentration under reduced pressure afforded 32 (62 mg, 85%) as a mixture of α and β anomers which were not separated; syrup; $|\alpha|_D^{20}$ -202° (c 1.12, chloroform); IR (film); 3600-3300 (OH, NH), 1750-1720 cm^{-1} (C=O, amide); MS (DCI/NH_3); m/z 503 ($M+NH_4^+$, base peak), 260, 243, 130 and 113.

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Anal. Calcd for $C_{20}H_{30}NO_9F_3$ (485.44): C, 49.48; H, 6.23; O, 29.66. Found: C, 49.52; H, 6.30; O, 29.80.

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