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A PREPARATIVE SYNTHESIS OF 3-AMINO-2,3,6-TRIDEOXY-L-*lyxo*-HEXOSE (DAUNOSAMINE) HYDROCHLORIDE FROM D-MANNOSE*†

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ABSTRACT

A simple, preparative route in nine steps from methyl α-D-mannopyranoside (**1**) is described that affords, in 40% overall yield, the title amino sugar **11**, the sugar constituent of the antitumor antibiotics adriamycin and daunorubicin. The 2,3:4,5-dibenzylidene acetal (**2**) of **1** is converted by butyllithium into the 2-deoxy-3-ketone **3**, whose oxime **4** is reduced with high stereoselectivity to the D-*ribo* amine, isolated as its *N*-acetyl derivative **5** and converted by action of *N*-bromosuccinimide into the 4-*O*-benzoyl-6-bromide **7**. Dehydrohalogenation of **7** gives the 5,6-unsaturated glycoside **8**, which, after *O*-debenzoylation to **9**, undergoes stereospecific reduction by hydrogen with net C-5 inversion to give the crystalline, *N*-acetylated methyl β-glycoside (**10**) of daunosamine, readily converted into daunosamine hydrochloride (**11**) and into the crystalline *N*-benzoyl (**14**) and *N*-acetyl (**15**) derivatives. No chromatographic procedures for isolation are required in any of the steps.

INTRODUCTION

Daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose, **11**) is the carbohydrate constituent of the anthracycline antibiotics daunorubicin[2–7] (daunomycin) and adriamycin[8–11]. Both of these antibiotics, adriamycin in particular, have shown promise as anticancer agents in clinical use[12,13]. The amino sugar **11** has been isolated from hydrolyzates of daunorubicin[5] and adriamycin[10], and it has been synthesized by a route starting from L-rhamnose (6-deoxy-L-mannose) by a group at the Stanford Research Institute[14].

In view of the high cost of microbially produced adriamycin, an effective synthetic route is a desirable objective; separate construction of the aglycon[15] and the sugar[14], followed by a coupling reaction[16], constitute a logical approach. As L-rhamnose is an expensive starting-sugar for preparation of daunosamine, and as the route described[14] requires several steps involving chromatographic resolution

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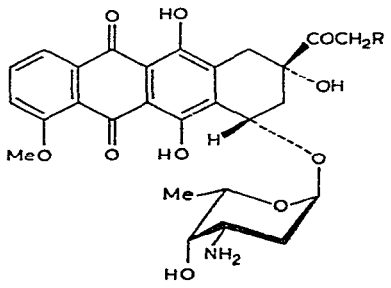


Daunorubicin R = H
Adriamycin R = OH

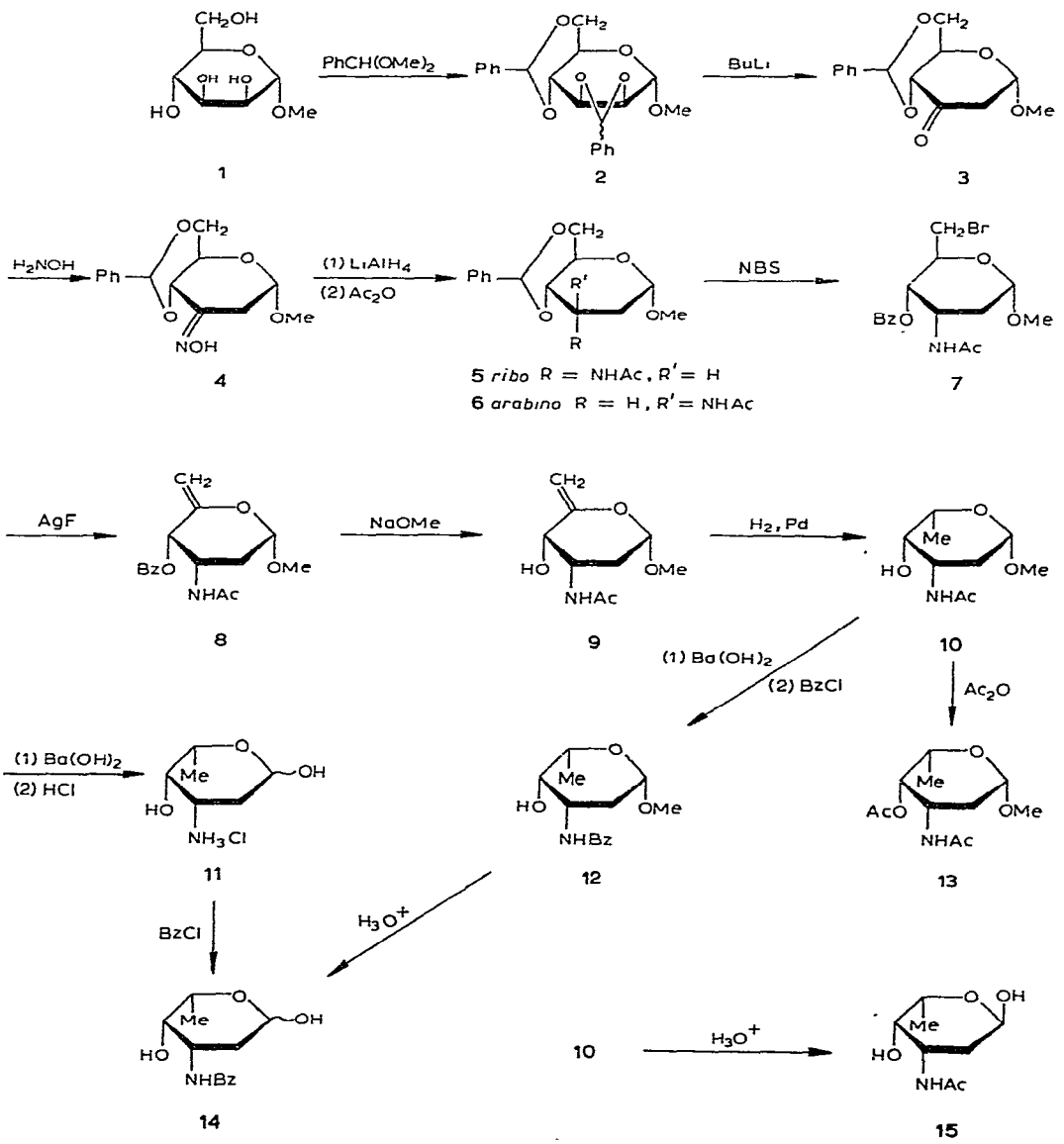
and gives **11** in only low overall yield, a convenient, high-yielding synthesis of **11** from an abundant sugar precursor would be a useful advance.

As part of a program on exploration of reactions useful for synthesis of biologically important sugars[17], we have examined various procedures for regio- and stereo-selective modification of carbohydrates. This report describes a high-yielding, preparative sequence for converting the abundant D-mannose into daunosamine (**11**); the major features of the route involve direct generation of a 2-deoxy-3-keto intermediate (**3**) whose oxime is reduced almost stereospecifically to introduce the correctly oriented 3-amino group, followed by a stereospecific step late in the sequence to introduce the terminal *C*-methyl group with inversion at C-5, to generate the required L-*lyxo* stereochemistry.

DISCUSSION

Methyl α-D-mannopyranoside (**1**) was benzylidenated with α,α-dimethoxytoluene by the general procedure of Evans[18] to give the 2,3:4,6-dibenzylidene acetal **2** in 95% yield as a crystalline mixture of products diastereoisomeric at the acetal position of the dioxolane ring. Purification to give a single diastereoisomer was not necessary for the next step in the sequence. Conversion of **2** into the key intermediate methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (**3**) was achieved by treatment with a 2-molar quantity of butyllithium in an adaptation of the very useful reaction of Klemer and Rodemeyer[19]. When attempted on a large scale, the procedure for conversion of **2** into **3**, reported[19] to give **3** in 54% yield on a small scale, led only to 3,5-*O*-benzylidene-1-deoxy-D-*erythro*-2-pentulose[20]. However, a minor modification (see Experimental section) permitted the successful large-scale conversion of **2** into **3**, isolated crystalline in 91% yield. The butyllithium reagent appears to attack the dioxolane ring of **2** specifically, with abstraction of the axially attached hydrogen atom (H-3) to generate the enolate anion of **3** with release of benzaldehyde (which reacts with a second molecule of the reagent); both of the dioxolane-ring isomers react to give the same product, but the reagent does not attack the 1,3-dioxane ring. Previously described routes[21–24] to compound **3** require many more steps, and the net yields are low.

Oximation of the ketone **3** by the procedure described in the literature[24] gave the crystalline oxime[24–26] **4** in >95% yield. Reduction of **4** with lithium aluminum hydride in ether, as described by Overend and co-workers[24] but with a procedural modification (see Experimental section) to permit large-scale adaptation (compound **4** has only low solubility in ether), gave a product that was acetylated directly to give a mixture containing 87% of the D-*ribo* product[27,28] **5** and 12% of the D-*arabino* diastereoisomer[26,27,29] **6**. These products were readily separated, without recourse to chromatography, by exploiting the very low solubility of **6** in toluene to separate



crystalline **6**, essentially quantitatively, from the mixture; the desired *ribo* product **5** was obtained quite free from **6**. Both **5** and **6** have been described previously[26–29], but were obtained by considerably more-tedious routes. The reduction of **4** by lithium aluminum hydride was reported by Overend and co-workers[24] as leading exclusively to the *ribo* amine; similar reduction of the acetylated oxime **4** followed by acetylation gave[26] a 1:3 mixture of **5** and **6**. This possibility of stereochemical control in the reduction of **4** is a useful feature of this synthesis if stereochemical analogues of daunosamine are desired.

Treatment of the *ribo* acetamido derivative **5** with *N*-bromosuccinimide in carbon tetrachloride, by the general procedure of Hanessian[30], led to opening of the 1,3-dioxane ring and formation of the 4-*O*-benzoyl-6-bromo-6-deoxy analogue **7**, isolated crystalline in 70% yield and characterized by elemental analysis, and i.r., n.m.r. (see Table I), and mass (see Table II) spectrometry. Dehydrobromination of **7** was effected with silver fluoride[31] by the established, general procedure[32], to afford the 5,6-unsaturated derivative **8** as a syrup in quantitative yield; the structure of this product was supported by microanalytical and spectral data (see Experimental section and Tables I and II). *O*-Deacylation of **8** was quantitatively effected by catalytic transesterification to give syrupy compound **9**, which underwent stereospecific hydrogenation in the presence of palladium-on-barium sulfate to give crystalline methyl 3-acetamido-2,3,6-trideoxy-β-L-*lyxo*-hexopyranoside (**10**, methyl *N*-acetyl-β-daunosaminide) in essentially quantitative yield. The structure assigned to **10** was supported by analytical and physical data (see Experimental section and Tables I and II) and by comparison with data for the known[33] β-D enantiomorph. Under the conditions used for the reduction of **9**, the reaction was fully stereospecific; none of the 5-epimer[20] of **10** was present in the product.

The final step in the sequence, conversion of **10** into the free amino sugar **11**, was most satisfactorily effected by *N*-deacetylating **10** with aqueous barium hydroxide and then hydrolyzing the resultant aminoglycoside with aqueous hydrochloric acid; 3-amino-2,3,6-trideoxy-L-*lyxo*-hexose (daunosamine) hydrochloride (**11**) was obtained in 84% yield as the crystalline α-L anomer. Its identity was confirmed by comparison with literature data[5,14,34] and by its X-ray powder diffractogram in comparison with that of an authentic sample kindly furnished by Dr. David W. Henry of the Stanford Research Institute.

The foregoing synthesis affords the amino sugar **11** from **1** in 9 steps with a net yield of 40%, based on intermediates isolated directly, and without recourse to chromatographic purification at any of the stages; further improvement in net yield could be effected by re-extraction of mother liquors at certain intermediate steps, and all steps are readily amenable to scaled-up operation.

A number of additional derivatives of the glycoside **10** and the final amino sugar **11** were also prepared. *N*-Deacetylation of **10** with barium hydroxide, as in the initial step used for converting **10** into **11**, followed by *N*-benzoylation with benzoyl chloride in buffered aqueous solution, gave the *N*-benzoyl analogue (**12**) of **10** as a crystalline solid; a side product isolated in this preparation proved to be 3-benzamido-

TABLE I

100-MHz N.M.R. SPECTRAL DATA FOR COMPOUNDS **2–10, 12, AND 13**

Compound[a]	*Chemical shifts (δ)*[b] *(first-order couplings, Hz, in parentheses)*													
	H-1 ($J_{1,2e}$)	*H-2e* ($J_{1,2a}$)	*H-2a* ($J_{2e,2a}$)	*H-3* ($J_{2e,3}$)	*H-4* ($J_{4,5}$)	*H-5* ($J_{5,6}$)	*H-6* ($J_{5,6'}$)	*H-6'* ($J_{6,6'}$)	*Aryl*	*NH*[c] ($J_{3\,NH}$)	*PhCH*	*OMe*	*Ac*	*OH*
2[d]	5.07d (2.8) 5.00d (2.0)	←4.80–3.60m→							7.4m		5.95s 5.49s 6.27s 5.61s	3.39s 3.37s		
3	5.09d (2.0)	2.58dd (5.0)	2.80dd (15.0)		←4.50–3.70m→				7.4m		5.55s	3.34s		
4	4.82dd (1.0)	3.48dd (5.0)	2.12dd (15.0)		←4.35–3.60m→				7.3m		5.54s	3.28s		8.95s (broad)
5	4.8	←~2→		←4.50–3.20m→					7.35m	6.65d	5.62s	3.37s	1.99s	
6[e]	4.74dd (1.0)	2.44m (3.3)	1.65m (13.5)	←4.50–3.40m→ (4.8)					7.62m	5.46d	5.53s	3.40s	1.95s	
7[f]	4.88–4.68m (4.0)	2.12m	1.85m (15.0)	4.88–4.68m (4.0)	4.97dd (9.8)	4.16m (2.9)	3.58dd (7.3)	3.41dd (11.4)	7.50m	6.80d (8.2)		3.46s	1.90s	
8	4.9–4.6m	2.22m	1.97m (15.0)	4.9–4.6m	5.57m		3.86	3.41	7.65m	6.63d (10.0)		3.47s	1.90s	
9[g]	4.61dd (4.0)	←2.10–1.60m→ (6.0)		←4.70–3.80m→						7.46d (8.0)		3.42s	1.83s	5.30 (broad)
10[g]	4.34dd (4.8)	←1.60–1.40m→ (7.3)		3.80m (broad)	3.29m (1.2)	3.48m (6.5)	←1.11d→			6.61d (8.2)		3.34s	1.79s	4.65d (6.3)
12[h]	4.40dd (2.5)	2.05m (9.5)	1.59m (14.8)	4.22m (broad) (5.0)	3.51m (1.0)	3.61m (6.5)	←1.27d→		7.56m	6.81d (9.0)		3.47s		2.68
13[i]	4.43dd (2.3)	~2 (9.3)	1.63m	4.22m	5.02dd (1.5)	3.69m (6.5)	←1.19d→			5.89d (7.8)		3.48s	2.15s 1.93s	

[a]In chloroform-*d*, unless otherwise stated. [b]Signal multiplicities: d, doublet; m, multiplet; s, singlet. [c]Broadened signal. [d]Two diastereoisomers present. [e]90-MHz Fourier-transform spectrum; compound had very low solubility in chloroform-*d*. [f]$J_{3,4}$ 4.4 Hz. [g]In methyl sulfoxide-d_6. [h]$J_{2a,3}$ 13 Hz. [i]$J_{3,4}$ 3 Hz.

2,3,6-trideoxy-L-*lyxo*-hexose (**14**, *N*-benzoyldaunosamine), evidently arising from partial hydrolysis of the very acid-labile glycoside **12** during a demineralization step involving use of an acidic, ion-exchange resin. Analytical and spectral data (see Experimental section and Tables I and II) for **12** were in full support of the structure assigned.

TABLE II

MASS-SPECTRAL DATA FOR COMPOUNDS **2–10, 12, 13, AND 15**

m/e *of principal fragments* (% *of base peak*)							*Assignment*
Compound							
2	**3**	**4**	**5**	**6**			
371 (11)	265 (1)	280 (1)	308 (3)	308 (1)			M+1
370 (33)	264 (4)	279 (4)	307 (10)	307 (4)			$M^{\ddagger}$
369 (9)	263 (5)	278 (2)	306 (3)	306 (3)			M−1
339 (3)	233 (9)	248 (16)	276 (11)	276 (21)			M−·OMe
	204 (1)	219 (16)	247 (2)				$M-HCO_2Me$
293 (1)		202 (2)					$M-C_6H_5\cdot$
264 (2)		173 (4)	201 (3)	201 (52)			M−PhCHO
221 (6)	115 (13)	130 (43)	158 (11)	158 (26)			h_1^a
149 (37)	149 (60)	149 (100)	149 (6)	149 (12)			h_2^a
106 (17)	106 (11)	106 (12)	106 (29)	106 (29)			$PhCHO^{\ddagger}$
105 (100)	105 (72)	105 (50)	105 (100)	105 (100)			$PhCO^+$
91 (60)	91 (70)	91 (58)	91 (30)	91 (29)			$PhCH_2^+$
77 (35)	77 (29)	77 (34)	77 (28)	77 (23)			Ph^+
	87 (100)						
Compound							
7	**8**	**9**	**10**	**12**	**13**	**15**	
387 (0.2)	306 (3)	202 (1)		266 (0.1)	246 (0.4)	190 (0.2)	M+1
386 (0.5)	305 (15)	201 (4)	203 (0.7)	265 (0.8)	245 (0.5)	189 (0.2)	$M^{\ddagger}$
385 (0.2)			202 (0.5)	264 (0.1)	244 (2)	188 (0.1)	M−1
354 (3)	273 (0.5)	169 (43)	171 (20)	233 (40)	213 (1)	171 (8)	A_1^b
232 (0.8)	151 (3)	151 (1)	153 (7)	215 (1.6)	153 (28)	153 (4)	A_2^b
138 (22)			138 (2)	200 (0.9)	138 (6)	138 (1)	A_3^b
263 (12)	183 (3)	183 (0.5)	185 (2)	247 (1.2)	185 (9)	171 (8)	B_1^b
205 (2)	140 (2)	140 (10)	142 (3)	142 (1.2)	142 (2)	128 (5)	B_2^b
355 (1)	274 (2)	170 (10)	172 (2)	234 (1)	214 (12)	172 (2)	C_1^b
296 (0.4)	215 (3)	111 (17)	113 (3)	113 (1)	155 (1)	113 (1.5)	C_2^b
174 (0.3)			95 (3)	95 (0.5)	95 (6)	95 (1)	C_3^b
263 (12)	263 (0.3)	159 (0.5)	159 (7)	221 (0.8)	201 (3)	145 (9)	D_1^b
205 (2)	205 (1)	101 (44)	101 (87)	163 (17)	143 (28)	101 (20)	D_2^b
163 (0.4)	163 (0.4)	59 (100)	59 (100)		101 (80)	59 (40)	$D_2-CH_2CO^b$
105 (100)	105 (100)			105 (100)			$PhCO^+$
77 (46)	77 (20)			77 (20)			Ph^+
43 (32)	43 (18)	43 (100)	43 (62)		43 (100)	43 (80)	Ac^+

[a]Fragment notation according to Chizhov *et al.* (see ref. 41). [b]Fragment assignments as proposed by Vigevani *et al.* (see ref. 42); the mass spectrum of compound **14** was found in good accord with that reported in ref. 42.

Peracetylation of the *N*-acetylated glycoside **10** with acetic anhydride–pyridine gave the corresponding 4-*O*-acetyl derivative **13** as needles (see Experimental section and Tables I and II for details of characterization). The α-L analogue of **13** has been described[34] in the literature; it has been obtained from daunosamine prepared from the natural antibiotic, and was contaminated with a small proportion of the β-L anomer **13**. In the n.m.r. spectrum published[34] for this anomeric mixture, the chemical shifts observed for the minor product correspond to those recorded here (see Table I) for compound **13**.

N-Benzoylation of daunosamine hydrochloride (**11**) with benzoyl chloride in buffered aqueous solution gave the crystalline, known 3-benzamido-2,3,6-trideoxy-L-*lyxo*-hexose (**14**), whose physical constants were in good agreement with those recorded for **14** obtained[5,34] from the natural amino sugar, and also correlated with the data recorded by Richardson[29] for the synthetic D enantiomorph. The same product (**14**) could also be readily prepared by mild hydrolysis, with aqueous acetic acid, of the corresponding methyl glycoside **12**.

The *N*-acetyl derivative (**15**) of daunosamine was readily prepared in high yield by hydrolysis of the methyl glycoside **10** with aqueous acetic acid. Compound **15** was obtained as the crystalline α-L enantiomorph, as indicated by its upward mutarotation; the final specific rotation observed showed fair correlation with that recorded[33] for the synthetic D enantiomorph.

Details of the n.m.r. and mass spectra of the products described in this paper, together with assignments, are recorded in Tables I and II.

EXPERIMENTAL

General methods. — Evaporations were performed under diminished pressure. Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. A Perkin–Elmer Model 141 polarimeter and 1-dm tubes were used for measurement of specific rotations. I.r. spectra were recorded with a Perkin–Elmer Model 457 grating i.r. spectrophotometer for potassium bromide pellets, unless otherwise indicated. N.m.r. spectra were recorded at 100 MHz with Varian HA-100 or JEOL MH-100 spectrometers; chemical shifts refer to an internal standard of tetramethylsilane ($\delta = 0.00$), and are recorded, together with spin-coupling values (Hz) in Table I. T.l.c. was performed with Silica Gel G (E. Merck, Darmstadt, Germany) activated at 120°. Solvent volumes are v/v; petroleum ether refers to the fraction boiling at 65–110°. Detection was by u.v. light and with sulfuric acid. Column filtrations were performed with silica gel (Merck No. 7734; 63–200 μm). Microanalyses were made by W. N. Rond. Mass spectra were recorded by C. R. Weisenberger with an AEI MS-9 double-focusing, high-resolution spectrometer operating at an ionizing potential of 70 eV and an accelerating potential of 8 kV; the source temperature (direct-inlet system) was 150°. Data and probable assignments are recorded in Table II. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. The camera diameter was 114.59 mm. Relative intensities were

estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

Preparation of methyl 2,3:4,6-di-O-benzylidene-α-D-mannopyranoside (**2**). — The following is an adaptation of the benzylidenation procedure used by Evans[18] with methyl glucopyranosides. A mixture of methyl α-D-mannopyranoside (**1**, 50 g, 258 mmoles), α,α-dimethoxytoluene (92 g, 600 mmoles), and anhydrous *p*-toluenesulfonic acid (1 g) in *N,N*-dimethylformamide (300 ml), in a 1-liter flask fitted with an air condenser attached to a water aspirator, was stirred magnetically and heated in an oil bath for 3 h at 65–75°. No starting material **1** remained after this time (t.l.c., 4:1 ether–petroleum ether). The mixture was poured with vigorous stirring into 1 liter of ice–water containing sodium hydrogen carbonate (30 g). The resultant precipitate was filtered off, resuspended in ice–water, filtered off again, and dried in air and finally *in vacuo* over phosphorus pentaoxide; yield 90.6 g (95%), m.p. 120–160°, $[\alpha]_D^{23}$ −33° (*c* 1, chloroform).

Anal. Calc. for $C_{12}H_{22}O_6$ (370.41): C, 68.10; H, 5.99. Found: C, 68.06; H, 6.04.

The product, sufficiently pure for the following step, appeared from its n.m.r. spectrum to be a diastereoisomeric mixture at the carbon atom of the 2,3-acetal. Recrystallization from propyl alcohol gave a product, having m.p. 180–182°, $[\alpha]_D$ 0° (*c* 1, chloroform), that behaved as a single isomer. For this compound, the following constants have been reported: m.p.[35] 178°; m.p.[36] 181–182°, $[\alpha]_D$ +0.03° (chloroform), yield of crude product 52.4%; m.p.[37] 176–177° (after recrystallization), yield 64.4%; m.p. [38] 174–178°.

Isolation and characterization of the two diastereoisomers will be reported separately[39].

Methyl 4,6-O-benzylidene-2-deoxy-α-D-erythro-hexopyranosid-3-ulose (**3**). — A large-scale adaptation of the procedure of Klemer and Rodemeyer[19] gave only traces of **3**, and afforded, instead, mainly 3,5-*O*-benzylidene-1-deoxy-D-*erythro*-2-pentulose[20]. The following modification gives the desired ketone **3** in excellent yields in scaled-up preparations. A solution of the diastereoisomeric mixture of acetals **2** from the preceding preparation (20 g, 54 mmoles) in commercial abs. tetrahydrofuran (400 ml) under nitrogen was cooled to −40°. Butyllithium in hexane (2.4M, 50 ml, 120 mmoles) was added, and the temperature was kept for 0.5 h below −30°, during which time the color of the solution turned from yellow to red and all of the starting material disappeared, as indicated by t.l.c. monitoring. T.l.c. plates were developed with 1:1 ether–petroleum ether, and the developed plates were heated *in vacuo* for 15 min at 125° before being sprayed with sulfuric acid for zone detection. The heating step was required for removal of 1-phenyl-1-pentanol, whose R_F value is the same as that of **2**. The solution, still at −30° or below, was then poured with vigorous mechanical stirring into ice–water (400 ml) containing ammonium chloride (50 g). Without separation of the layers, the tetrahydrofuran was removed on a rotary evaporator at a bath temperature of ~30°. The aqueous slurry remaining was cooled to 0°, and the crystalline deoxy ketone **3** was filtered off with use of suction and dried;

yield 13 g (49 mmoles, 91%). This product could be used without further purification for the oximation to give **4**.

Recrystallization from ethanol gave pure **3**, m.p. 170–171°, $[\alpha]_D^{22}$ +150° (*c* 1, ethyl acetate) (lit.[19] m.p. 170–171°, $[\alpha]_D$ +153° in ethyl acetate); ν_{max}^{KBr} 1740 (C=O), 745 and 695 cm^{-1} (aryl); X-ray powder diffraction data: 13.18 vw, 9.71 m, 8.58 m, 7.56 vw, 6.83 m, 5.66 m, 5.26 w, 4.86 vw, 4.51 w, 4.18 s (2), 3.77 vs (1), and 3.46 m (3).

The product was indistinguishable from an authentic sample (by i.r., n.m.r., and mass spectrometry).

*Methyl 4,6-*O*-benzylidene-2-deoxy-α-*D*-*erythro*-hexopyranosid-3-ulose oxime* (**4**). — The deoxy ketone **3** (25 g, 94.7 mmoles) was oximated by the procedure described by Overend and colleagues[24] to afford the oxime **4**; yield 26 g (98.5%), m.p. 208° (from ethanol), $[\alpha]_D^{23}$ +202° (*c* 1.2, chloroform) (lit.[24] m.p. 207–208°, $[\alpha]_D$ +200° in chloroform; m.p. 203°, $[\alpha]_D$ +172° in chloroform[25]; and m.p. 208–209°, $[\alpha]_D$ +170° in chloroform[26]); ν_{max}^{KBr} 3330 (OH), 1670 (C=N), 750 and 695 cm^{-1} (aryl); X-ray powder diffraction data: 8.84 w, 8.22 m, 6.70 s (3,3), 5.69 m, 5.29 s (3,3), 4.50 m, 4.11 vs (1), 3.70 s (2), 3.20 vw, and 3.15 m.

*Methyl 3-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-*D*-*ribo*-hexopyranoside* (**5**) *and its* α-D-arabino *analogue* (**6**). — In a 2-liter flask equipped with a magnetic stirrer, a Soxhlet extractor, and a reflux condenser was placed lithium aluminum hydride (12 g, 316 mmoles) in ether (1 liter); and in the extractor thimble was placed the oxime **4** (23.5 g, 84.3 mmoles). The contents of the flask were stirred and heated under reflux for 24 h, after which time the excess of the reducing agent was decomposed[40] by successively adding water (12 ml), 15% aqueous sodium hydroxide (12 ml), and water (36 ml). The resultant mixture was filtered, and the filtrate evaporated to give a crystalline residue; this was dissolved in pyridine (140 ml), and acetic anhydride (70 ml) was added, with cooling to 0°. After 18 h at ~25°, the solution was poured into ice–water (600 ml), and the product extracted with dichloromethane (3 × 100 ml). The extract was successively washed with aqueous sodium hydrogen carbonate and water, dried (magnesium sulfate), and evaporated *in vacuo*. Pyridine (two 50-ml portions) and toluene (two 50-ml portions) were successively added to and evaporated from the residue. To the semicrystalline residue resulting was added toluene (120 ml), and the mixture was cooled to 0°. The crystalline precipitate was then filtered off with suction, and washed with a small volume of cold toluene, to afford the *arabino* derivative **6**; yield 3.2 g (12.3%). Recrystallization from acetone gave long needles, m.p. 272° (sublimation), $[\alpha]_D^{23}$ +68° (*c* 0.7, chloroform) (lit.[27] m.p. 274–277°, $[\alpha]_D$ +64.4° in chloroform; m.p. 284–285°, $[\alpha]_D$ +64° in ethanol[26]; and[29] m.p. 272–274°, $[\alpha]_D$ +65° in chloroform); ν_{max}^{KBr} 3270 (NH), 1650, 1565 (NHCO), 745 and 695 cm^{-1} (aryl); X-ray powder diffraction data: 15.22 w, 10.21 s, 7.69 m, 5.30 s, 5.06 w, 4.64 s (3), 4.41 s (2), 4.12 vs (1), and 3.77 w.

The mother liquor was evaporated to give the syrupy *ribo* derivative **5**; yield 22.5 g (87%), $[\alpha]_D^{23}$ +60° (*c* 1, chloroform) (lit.[27] $[\alpha]_D$ +49.8° in chloroform, and[28] $[\alpha]_D$ +56° in chloroform); ν_{max}^{KBr} 3410 (NH), 1660, 1510 (NHCO), 755 and 700 cm^{-1} (aryl).

The products **5** and **6** had R_F values of 0.64 and 0.59, respectively, in t.l.c. with 4:1 benzene–ethanol, and it was verified that each product was free from contamination by the other.

*Methyl 3-acetamido-4-O-benzoyl-6-bromo-2,3,6-trideoxy-α-*D-ribo-*hexopyranoside* (**7**). — To a solution of compound **5** (16 g, 52 mmoles) in dry carbon tetrachloride (400 ml) were added *N*-bromosuccinimide (11 g, 61.8 mmoles) and barium carbonate (15 g). The mixture was boiled under reflux for 2 h under normal room-illumination, during which time the mixture, originally colorless, became successively yellow, red, and, finally, faintly yellow. The solvent was removed *in vacuo*, and the residue was extracted with dichloromethane (200 ml); the clear extract was washed successively with 5% aqueous sodium hydrogen sulfite and aqueous sodium hydrogen carbonate, dried (magnesium sulfate), and evaporated. The resultant, crystalline residue was recrystallized from ethanol to give analytically pure **7**; yield 14 g (70%), m.p. 173°, $[\alpha]_D^{22}$ +76.5° (*c* 1, chloroform); ν_{max}^{KBr} 3400 (NH), 1735 (ester C=O), 1675 and 1535 cm^{-1} (NHCO); X-ray powder diffraction data: 14.36 m, 7.40 m (3), 6.91 w, 5.30 m (2), 5.02 w, 4.39 s (1), 3.34 w, 3.16 w, 3.11 w, and 2.89 vw.

Anal. Calc. for $C_{16}H_{20}BrNO_5$ (386.25): C, 49.76; H, 5.22; Br, 20.69; N, 3.63. Found: C, 49.60; H, 5.52; Br, 20.41; N, 3.81.

*Methyl 3-acetamido-4-O-benzoyl-2,3,6-trideoxy-α-*D-erythro-*hex-5-enopyranoside* (**8**). — A mixture of compound **7** (5 g, 13 mmoles) and dry, technical-grade silver fluoride[31] (5 g, 22.1 mmoles) in dry pyridine (90 ml) was stirred for 14 h at ~25°, after which time, t.l.c. (2:3 benzene–acetone) showed that all of the **7** had reacted. The dark solution was poured into ether (500 ml), and the resultant mixture was filtered. The filtrate was evaporated at ⩽40°, and then three 25-ml portions of toluene were added to and evaporated from the residue (to remove all of the pyridine). The resultant syrup was taken up in ether, and the suspension filtered through a small column (250 × 20 mm) of silica gel to remove residual silver salts. The effluent was evaporated *in vacuo* to give the pure derivative **8** as a syrup; yield 4 g (100%), $[\alpha]_D^{23}$ +55.2° (*c* 1.4, chloroform); ν_{max}^{film} 3420–3290 (NH), 1725 (ester CO), 1660 (Amide I, C=C), 1600, 1585 (monosubstituted phenyl), and 1525 cm^{-1} (Amide II).

Anal. Calc. for $C_{16}H_{19}NO_5$ (305.33): C, 62.94; H, 6.27; N, 4.56. Found: C, 62.52; H, 6.52; N, 4.21.

*Methyl 3-acetamido-2,3,6-trideoxy-α-*D-erythro-*hex-5-enopyranoside* (**9**). — To a solution of compound **8** (5 g, 16.4 mmoles) in abs. methanol (30 ml) was added M sodium methoxide (0.5 ml), and the mixture was kept for ~12 h at ~25°, at which point, t.l.c. (2:3 benzene–acetone) indicated that saponification was complete. The solution was passed through a small bed (250 × 20 mm) of silica gel in a column, and the effluent was evaporated *in vacuo* to give **9** as a syrup, yield 3.2 g (97%); this was subjected, without delay, to the hydrogenation reaction described next.

To secure an analytical sample free from methyl benzoate, the syrup was dissolved in water, the solution was washed twice with dichloromethane, and the aqueous solution was freeze-dried, giving pure **9**; $[\alpha]_D^{22}$ +74.5° (*c* 1, water); ν_{max}^{film} 3500–3200 (OH, NH), 1655 (Amide I, C=C), and 1525 cm^{-1} (Amide II).

*Methyl 3-acetamido-2,3,6-trideoxy-β-*L*-lyxo-hexopyranoside* (**10**) (*methyl* N-*acetyl-β-daunosaminide*). — A solution of the unsaturated sugar **9** (1.5 g, 7.46 mmoles) in abs. methanol (50 ml) was hydrogenated in the presence of 10% palladium-on-barium sulfate (150 mg) at atmospheric pressure. After 30 min, the theoretical amount of hydrogen had been taken up, and t.l.c. (2:3 benzene–acetone) verified that the reaction was complete. The catalyst was filtered off, and the filtrate was evaporated *in vacuo* to give a crystalline, chromatographically homogeneous residue of **10**; yield 1.5 g (99%). For analytical purposes, a sample was recrystallized from ethyl acetate to give **10** as fine needles; m.p. 208–210° (sublimation), $[\alpha]_D^{22}$ −28° (*c* 1, water); ν_{max}^{KBr} 3440, 3280 (OH, NH), 1630 and 1530 cm^{-1} (amide); X-ray powder diffraction data: 9.30 s (2,2), 7.37 m, 6.10 s (2;2), 5.08 vw, 4.68 m, 4.34 vs (1), 3.88 m (4), 3.71 s (3), 3.03 m, 2.87 m, 2.80 m, 2.54 m, 2.40 m, 2.28 m, 2.13 m, and 2.05 w.

Anal. Calc. for $C_9H_{17}NO_4$ (203.24): C, 53.19; H, 8.43; N, 6.89. Found: C, 53.15; H, 8.68; N, 6.81.

For the β-D enantiomorph of **10**, Baer and coworkers reported[33] m.p. 210–212°, and $[\alpha]_D$ +27.7° in water.

Compound **10** could be clearly resolved by t.l.c. from its 5-epimer (the D-*ribo* analogue[20] of **10**), but no trace of the 5-epimer was found in **10** or in the mother liquor from the crystallization. Comparable yields of **10** were obtained in scaled-up adaptations of the procedure described.

*3-Amino-2,3,6-trideoxy-*L*-lyxo-hexose* (*daunosamine*) *hydrochloride* (**11**). — A solution of the *N*-acetylated glycoside **10** (300 mg, 1.48 mmoles) and barium hydroxide octahydrate (946 mg, 3 mmoles) in water (6 ml) was boiled for 12 h under reflux, after which time, t.l.c. (2:3 benzene–acetone) revealed only a trace of the starting glycoside **10** (R_F 0.36) accompanying the *N*-deacetylated product (R_F 0.1). Solid carbon dioxide was added, and the resultant precipitate of barium carbonate was filtered off with suction. The filtrate was then lyophilized to give a solid that was dissolved in abs. ethanol and freed from traces of inorganic material by filtration. The filtrate was evaporated to dryness, and the residue dissolved in 0.5M hydrochloric acid (10 ml). The solution was heated for 3 h at 100°, decolorized with activated charcoal, and lyophilized to give a foam that readily crystallized on addition of acetone (4 ml). The crystals were filtered off in an inert atmosphere (nitrogen) and dried; yield 200 mg (84%), m.p. 168–170° (dec.), $[\alpha]_D^{23}$ −65.4° (equil., *c* 1.3, water); X-ray powder diffraction data: 7.43 s (2), 7.02 w, 4.54 m (3), 4.18 vs (1), 3.73 m, 3.52 m, and 3.32 m.

The m.p. of the product was undepressed on admixture with an authentic sample, and an authentic sample showed the same X-ray diffraction lines as those recorded here. The two samples also showed identical i.r. spectra.

For this compound, the following constants have been recorded: m.p. 168° (dec.), $[\alpha]_D$ −55.4° in water[5]; m.p. 160° (dec.), $[\alpha]_D$ −54.2° in water[14]; m.p. 168°, $[\alpha]_D$ −55.4° in water[34]; and (for the D enantiomorph), syrup, $[\alpha]_D$ +63.7° in water[33].

*Methyl 3-benzamido-2,3,6-trideoxy-β-*L*-lyxo-hexopyranoside* (**12**). — A solution of compound **10** (300 mg, 1.48 mmoles) and barium hydroxide octahydrate (946 mg,

3 mmoles) in water (10 ml) was boiled for 24 h under reflux, whereupon t.l.c. (see preceeding experiment) showed that **10** was absent. After neutralization as in the preceding experiment, potassium hydrogen carbonate (2.5 g) in water (10 ml) was added to the filtrate, and the solution was cooled to 0°. A cold solution of benzoyl chloride (1 ml) in acetone (10 ml) was then added, and the mixture was stirred for 3 h at 0° and then 18 h at ~25°. A single product (R_F 0.8, 2:3 benzene–acetone) was indicated by t.l.c. at this stage but, after demineralization of the solution by stirring with 30 ml of a 1:1 mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) ion-exchange resins, a second, minor product (R_F 0.55) was detected. The mixture was purified by preparative t.l.c. with the foregoing solvent, to give **12** as the main product, which was recrystallized from ether as needles; yield 140 mg (36%), m.p. 183–184°, $[\alpha]_D^{23}$ −10.5° (*c* 1, chloroform); ν_{max}^{KBr} 3410 (OH), 3250 (NH), 1610 and 1535 (NHCO), and 1575 cm^{-1} (monosubstituted benzene); X-ray powder diffraction data: 12.10 s (2), 8.30 vw, 6.04 s (1), 5.32 w, 4.68 m, 4.10 s (3), 3.96 w, 3.74 w, and 3.37 w.

Anal. Calc. for $C_{14}H_{19}NO_4$ (265.312): C, 63.38; H, 7.22; N, 5.28. Found: C, 63.13; H, 7.32; N, 5.34.

The minor product was obtained crystalline from acetone; yield 30 mg (8%), and was found indistinguishable from 3-benzamido-2,3,6-trideoxy-L-*lyxo*-hexose (**14**), described elsewhere in this paper.

*Methyl 3-acetamido-4-O-acetyl-2,3,6-trideoxy-β-*L-lyxo-*hexopyranoside* (**13**). — Compound **10** (300 mg, 1.48 mmoles) was treated with 1:2 acetic anhydride–pyridine (7.5 ml) for 18 h at ~25°. The mixture was poured into ice–water, extracted with dichloromethane, and the extract washed successively with aqueous sodium hydrogen carbonate and water, dried (magnesium sulfate), and evaporated *in vacuo*. Pyridine (two 10-ml portions) and then toluene (two 10-ml portions) were added to and evaporated from the residue, and then the crystalline residue was recrystallized from ether to give **13** as needles (150 mg, 41.5%), m.p. 203° (sublimation), $[\alpha]_D^{24}$ −27° (*c* 1, chloroform); ν_{max}^{KBr} 3310 (NH), 1740 (ester CO), 1650 and 1535 cm^{-1} (amide); X-ray powder diffraction data: 11.18 m, 9.35 vw, 7.82 s (2), 7.16 m, 5.69 m, 4.86 vs (1), 4.47 w, 4.27 w, and 3.81 m.

Anal. Calc. for $C_{11}H_{19}NO_5$ (245.28): C, 53.87; H, 7.81; N, 5.71. Found: C, 53.58; H, 7.58; N, 5.97.

The product proved to be somewhat water-soluble, and some loss of material evidently occurred during washing of the organic solution.

*3-Benzamido-2,3,6-trideoxy-*L-lyxo-*hexose* (**14**) (N-*benzoyldaunosamine*). — *A. From daunosamine hydrochloride* (**11**). To a cold (0°) solution of **11** (300 mg, 1.64 mmoles) and potassium hydrogen carbonate (2.5 g) in water (10 ml) was added a cold solution of benzoyl chloride (1 ml) in acetone (10 ml), and the mixture was stirred for 3 h at 0° and 18 h at ~25°. T.l.c. (2:3 benzene–acetone) then showed a major product (R_F 0.56) together with two faster-migrating components. The solution was de-ionized with a mixture of Amberlite IR-120 (H^+) and IRA-400 (OH^-) resins, lyophilized, and the residue purified by preparative t.l.c. with the foregoing solvent mixture to give **14** as a syrup that crystallized readily from acetone; the crystals were

filtered off, and washed with a little ethyl acetate; yield 200 mg (48.5%), m.p. 151–153°, $[\alpha]_D^{23}$ −107° (*c* 0.5, ethanol) (lit.[5] m.p. 154–156°, $[\alpha]_D$ −107.5° in ethanol; m.p. 151–152°, $[\alpha]_D$ −107.5° in ethanol[34]; and m.p. 150.5–151.5°, $[\alpha]_D$ +110° in ethanol for the D enantiomorph[29]); ν_{max}^{KBr} 3430, 3310 (OH, NH), 1630, 1525 (NHCO), and 1575 cm^{-1} (monosubstituted phenyl); X-ray powder diffraction data: 13.28 m, 11.86 w, 10.77 m, 8.97 s (3,3), 7.96 vw, 6.58 s (2), 5.96 vw, 5.62 vs (1), 4.86 m (broad), 4.63 m, 4.44 m, 4.29 m, 4.08 s (3,3), 3.93 m, and 3.77 m.

*B. From methyl 3-benzamido-2,3,6-trideoxy-β-*L*-lyxo-hexopyranoside* (**12**). A solution of compound **12** (50 mg, 189 μmoles) in acetic acid (1 ml) and water (5 ml) was boiled for 20 min under reflux, whereupon t.l.c. (see preceding part *A*) indicated that hydrolysis of the glycoside was complete. Evaporation gave a residue that crystallized readily on addition of a little acetone, and the crystals were filtered off with the aid of ethyl acetate, to give **14** (25 mg, 53%) indistinguishable from the sample prepared by route *A*.

*3-Acetamido-2,3,6-trideoxy-α-*L*-lyxo-hexose* (**15**) (N-*acetyl-α-daunosamine*). — A solution of the *N*-acetylated glycoside **10** (200 mg, 985 μmoles) in water (5 ml) and acetic acid (2 ml) was boiled for 30 min under reflux, whereupon t.l.c. (10:1 chloroform–methanol) showed that **10** (R_F 0.5) had all been converted into the product **15** (R_F 0.1). The solution was evaporated, and the residue recrystallized from ethyl acetate to give compound **15**; yield 150 mg (80%); m.p. 162°, $[\alpha]_D^{23}$ −180 (initial, extrapolated) → −116 (6 min) → −100° (30 min, equil., *c* 0.55, water); ν_{max}^{KBr} 3430, 3310 (OH, NH), 1625 and 1550 cm^{-1} (NHCO); X-ray powder diffraction data: 9.98 vw, 8.54 vs (1), 7.02 m, 5.48 w, 4.53 s (3), 4.23 s (2), 4.05 vw, and 3.86 w.

Anal. Calc. for $C_8H_{15}NO_4$ (189.21): C, 50.78; H, 7.99; N, 7.40. Found: C, 50.93; H, 7.87; N, 7.34.

The D enantiomorph has been reported[33] to have m.p. 145–146° and 151–153°, $[\alpha]_D$ +101 → +94.2° in water.

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