PREPARATION OF 2-AMINO-2,4-DIDEOXY-D-lyxo-HEXOPYRANOSE (4-DEOXY-D-MANNOSAMINE) FROM 1,6-ANHYDRO-β-D-GLUCOPYRANOSE*

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1,6-Anhydro-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-*xylo*-hexopyranose (*III*), obtainable by a four-step synthesis from 1,6-anhydro-β-D-glucopyranose, was converted to 3-O-(N-benzylcarbamoyl) derivative *V* on reaction with benzyl isocyanate. The cyclization of *V* with sodium hydride in dimethylformamide gave a derivative of oxazolidin-2-one, *VII*, which on alkaline hydrolysis and hydrogenolysis gave the hydrochloride of 2-amino-1,6-anhydro-2,4-dideoxy-β-D-*lyxo*-hexopyranose (*XI*). When 1,6-anhydro derivative *XI* was treated with hydrochloric acid the hydrochloride of 2-amino-2,4-dideoxy-D-*lyxo*-hexopyranose (*XIV*) was obtained. Corresponding N-acetyl derivative *XVI* is suitable for studies of enzymatic synthesis of N-acetylneuraminic acid.

N-acetylneuraminic acid (NeuAc)[1] is an important component of glyco-conjugates the study of which should contribute to further knowledge of the mechanism of cellular phenomena on a molecular level. One of the possible approaches to the study of the role of NeuAc is the affecting of its biosynthesis from hexosamines by modifying the starting hexosamine molecule in a way which leads either to inhibition, or the biosynthesis of correspondingly modified NeuAc. We consider that 4-deoxy-D-mannosamine could be interesting for studies of the mentioned type, since the substitution of the 4-hydroxyl group by hydrogen does not lead to a conformation change and it decreases the polarity of the molecule only indistinctly. As the hydroxyl group on $C_{(6)}$ is not substituted, it can be phosphorylated, and the phosphorylation often represents the first step in the biochemical cycles of saccharides. So far 6-fluoro[2] and 6-azido derivative[3] of 2-acetamido-2,6-dideoxy-D-mannose and some N-acyl and N-alkyl derivatives of D-glucosamine and D-mannosamine[4,5] were used for similar studies.

The synthesis of 2-amino-2,4-dideoxy-D-*lyxo*-hexopyranose (4-deoxy-D-mannosamine) is based on the earlier elaborated method of substitution of the tosyloxy group

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by the amino group on $C_{(2)}$ in 1,6-anhydro-β-D-glucopyranose, thus opening the way to the preparation of 2-amino-2-deoxy-D-mannoses modified at $C_{(4)}$ (ref.[6]). As a starting compound 1,6-anhydro-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-*xylo*-hexopyranose (*III*) was used, which can be prepared by direct reduction of 1,6:3,4-dianhydro-2-O-*p*-toluenesulfonyl-β-D-galactopyranose (*I*) with Raney nickel[7]. However, during this reduction the catalyst is partly deactivated and the reaction is not always accomplished, which – together with the formation of a small amount of by-products – decreases the yield. Therefore preference was given to a two-step procedure, consisting in the conversion of epoxide *I* to iodo derivative *II* and its hydrogenolysis on palladium on charcoal. The opening of epoxide *I* with hydrogen iodide in chloroform took place at 0°C within a few mintues, in a quantitative yield. The use of hydrogen iodide in dioxane[8] or of sodium iodide with ammonium fluoride led to lower yields[9], while methyl iodide under catalysis with boron trifluoride etherate[9] gave a 90% yield.

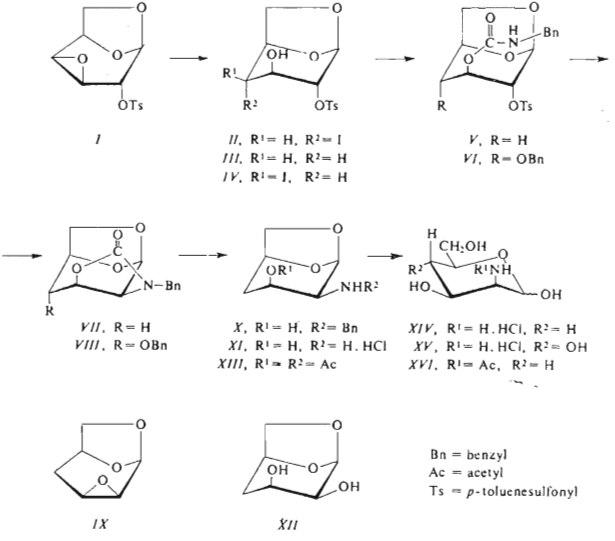
During the hydrogenolysis of the iodo derivative *II* on palladium on charcoal in ethanol in the presence of sodium hydrogen carbonate a partial isomerization of the starting D-*gluco* derivative *II* to D-*galacto* derivative *IV* took place. The latter can be isolated during the initial phases of the reaction. The structure of D-*galacto* derivative *IV* was determined by means of ^{1}H NMR spectroscopy, a comparison of its properties with those given in literature[8], and also follows from its formation in the reaction of iodine derivative *II* with potassium iodide in dimethylformamide. The end product, deoxytosyl ester *III*, obtained on hydrogenolysis of both isomers, *II* and *IV*, was isolated in a practically quantitative yield.

Ester *III* was converted to urethane *V* by five hours boiling with benzyl isocyanate in toluene. The cyclization of urethan *V* to the oxazolidin-2-one derivative *VII* took place less selectively than the similar conversion of urethan *VI* to derivative *VIII*, which was used in the preparation of 2-amino-1,6-anhydro-2-deoxy-β-D-mannopyranose[6]. As a cyclization reagent sodium hydride in dimethylformamide was tested, which in the case of derivative *VI* gives oxazolidine *VIII* in 94% yield, as shown by the analysis of the reaction mixture by gas chromatography. However, in the case of derivative *V* epoxide *IX* is formed as a by-product to a higher extent; during a preparative experiment 56% of the oxazolidin-2-one derivative *VII* and 9% of epoxide *IX* were isolated.

When comparing the IR spectra of urethane *V* with the cyclic product *VII* an increase in the wave-number of the carbonyl group vibration can be observed, which is almost identical with a similar shift in derivatives with a benzyl group on $C_{(4)}$ (*VI* and *VIII*) and which corresponds to the difference in the carbonyl frequencies for ethyl wrethane and 1,3-oxazolidin-2-one[10] (Table I).

In the subsequent synthetic step hydrolysis of the oxazolidinone ring in derivative *VII* was carried out with sodium hydroxide in aqueous ethanol, and the benzylamino derivative *X* formed was hydrogenolysed on palladium on charcoal, thus eliminating

the protecting group. The molar optical rotation of the 2-amino-1,6-anhydro-2,4-dideoxy-β-D-*lyxo*-hexopyranose *XI* formed, $[M]_D$ −116° (water), is comparable with the rotation of the analogous nitrogen-less derivative, 1,6-anhydro-4-deoxy-β-D-*lyxo*-hexopyranose[11] (*XII*), $[M]_D$ −127°. Acetylation of hydrochloride *XI* with acetic anhydride in pyridine gave diacetyl derivative *XIII*, suitable for identification.



I

II, R¹ = H, R² = I
III, R¹ = H, R² = H
IV, R¹ = I, R² = H

V, R = H
VI, R = OBn

VII, R = H
VIII, R = OBn

X, R¹ = H, R² = Bn
XI, R¹ = H, R² = H.HCl
XIII, R¹ = R² = Ac

XIV, R¹ = H.HCl, R² = H
XV, R¹ = H.HCl, R² = OH
XVI, R¹ = Ac, R² = H

IX

XII

Bn = benzyl
Ac = acetyl
Ts = *p*-toluenesulfonyl

TABLE I

Comparison of the wave-numbers of the carbonyl vibrations in acyclic and cyclic urethanes

Acyclic derivative	Wave-number[a] ν(C=O)	Cyclic derivative	Wave-number[a] ν(C=O)	Difference[a] Δν
V	1 730	*VII*	1 756	26
VI	1 734	*VIII*	1 761	27
ethyl-urethane	1 689[b]	1,3-oxazolidin-2-one	1 724[b]	35

[a] In cm^{-1}; [b] from literature[10].

The parameters of the ^{1}H NMR spectrum of hydrochloride *XI* in deuterium oxide show that it exists in the $^1C_4$(D) conformation. The relatively small shift of H-2, $\delta = 3{\cdot}49$, confirms the presence of the amino group on $C_{(2)}$, while the pair of the multiplets with the shifts $\delta = 2{\cdot}03$ and $2{\cdot}29$, characterizes the arrangement at $C_{(4)}$. The coupling constants $J_{2,3} = 5{\cdot}4$, $J_{3,4a} = 4{\cdot}5$ and $J_{4a,5} = 4{\cdot}1$ Hz indicate a *cis* (*gauche*) arrangement of the hydrogens H-2, H-3, H-4a and H-5. The coupling constants $J_{3,4e} = 1{\cdot}7$ and $J_{4e,5} = 1{\cdot}8$ Hz correspond to the configuration of the hydrogen H-4e. The proton pair H-6_{endo} and H-6_{exo}, with the shifts $\delta = 4{\cdot}34$ and $3{\cdot}79$, forms multiplets ($J_{6,6} = -7{\cdot}3$, $J_{5,6endo} = 0{\cdot}9$, $J_{5,6exo} = 5{\cdot}4$ Hz) characteristic of the presence of a 1,6-anhydro bond. The coupling constants J of the ^{1}H NMR spectrum of hydrochloride *XI* are well comparable, as expected, with similar parameters[12] of the deoxy derivative *XII*, $J_{2,3} = 5{\cdot}0$, $J_{3,4a} = 4{\cdot}3$, $J_{3,4e} = 1{\cdot}7$, $J_{4a,5} = 4{\cdot}3$, $J_{4e,5} = 1{\cdot}7$, and they only display differences following from the substitution of the amino group on $C_{(2)}$ in compound *XI* for the hydroxyl group.

Acid hydrolysis of hydrochloride *XI* in azeotropic hydrochloric acid took place within 2 h at 100°C, without important degradation which usually accompanies reactions of this type. The hydrochloride of 2-amino-2,4-dideoxy-β-D-*lyxo*-hexopyranose (*XIV*) obtained crystallized after several months of standing. In aqueous solution it displays mutarotation during which equilibrium is attained practically within 30 min; $[\alpha]_D$ $-10{\cdot}8°$ (3 min) → $-1{\cdot}7°$ (30 min). In this it differs from the hydrochloride of 2-amino-2-deoxy-D-mannopyranose (*XV*), where the equilibrium attainment takes place so rapidly that mutarotation in water cannot be observed[13]. The composition of the equilibrium mixture of the anomers, *i.e.* 13% of α-anomer and 87% of β-anomer of hydrochloride *XIV*, determined on the basis of the ^{1}H NMR spectrum, differs distinctly from the mixture of the anomers of the hydrochloride of 2-amino-2-deoxy-D-mannose (*XV*) in which 37% of α-anomer and 63% of β-anomer were found (Horton[13] gives the values 43% for α-anomer and 57% for β-anomer, Botto[14] gives 40% and 60%, respectively). The difference in the behaviour of the two substances becomes still more evident when comparing them with the pair D-mannose -4-deoxy-D-*lyxo*-hexose (4-deoxy-D-mannose), where a insignificant difference in the ratio of the anomers was found (α : β = 67 : 33 for D-mannose and 62 : 38 for its 4-deoxy derivative[15]). A simple explanation of this phenomenon cannot be put forward so far, but it seems that the difference is not due to the existence of compounds *XIV* and *XV* in various conformations of the pyranose ring, because the parameters of the ^{1}H NMR spectra of both substances corresponds to the $^4C_1$(D) conformation (Table II).

Acetylation of hydrochloride *XIV* with acetic anhydride in water in the presence of an ion exchanger in carbonate form[16] gave N-acetyl derivative *XVI* which is an amorphous mixture of anomers. The N-acetylhexosamine *XVI* was used for preliminary experiments in a cell-free system from rate liver, synthetizing NeuAc, which showed its inhibitory ability[17].

EXPERIMENTAL

The melting points were measured on a micromelting point apparatus Boëtius, the optical rotation on a Bendix–Ericsson 134A polarimeter at 23—25°C. The IR spectra were measured in chloroform on a Zeiss-Jena UR-20 spectrophotometer. The wave-numbers of characteristic vibrations are given in cm^{-1}. The ^1H NMR spectra were measured on a Tesla BS 467 (60 MHz) and Varian XL-200 (200 MHz) instrument; chemical shifts are given in ppm in δ-scale, the coupling constant values J in Hz: Tetramethylsilane (TMS) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate DSS) were used as internal references. All the parameters were obtained by 1st order analysis, for assignments the method of double resonance was employed. Preparative chromatography was carried out on silica gel columns (Lachema, 60—120 μm), while silica gel G (Merck) was used for thin-layer chromatography (TLC). The solvent systems which were used for the monitoring of individual reactions by thin-layer chromatography are given in the text after the abbreviation TLC. For detection the universal detection by spraying with 50% sulfuric acid and mineralization by heating was used. Aminohexoses were detected specifically with 0·5% ninhydrin in ethanol and reducing sugars by spraying first with a 0·3% silver nitrate solution in moist acetone and then with a 2% solution of NaOH in 95% aqueous methanol. Gas chromatography was carried out on a Chrom 3 (Laboratory Apparatus) instrument, using nitrogen as carrier gas and flame ionization detector. Hydrogenations were carried out at atmospheric pressure, using water as sealing liquid. For the drying of solutions in non-polar solvents anhydrous magnesium sulfate was used. All solutions were evaporated on a vacuum rotatory evaporator at 40—50°C (bath temperature) and 2 kPa. The samples for analysis were dried over phosphorus pentoxide at 25 Pa.

1,6-Anhydro-4-deoxy-4-iodo-2-O-*p*-toluenesulfonyl-β-D-glucopyranose (*II*)

A solution of hydrogen iodide in chloroform (10%, 100 ml) was added to a stirred solution of epoxide *I* (30 g; 0·1 mol) in chloroform (150 ml) at 0°C. TLC: benzene–acetone (10 : 1). After

TABLE II

Selected parameters of the ^1H NMR spectra (200 MHz) of the hydrochlorides of 2-amino-2,4-dideoxy-D-*lyxo*-hexopyranose (*XIV*) and 2-amino-2-deoxy-D-mannopyranose (*XV*) in deuterium oxide

Parameter	*XIV*		*XV*	
	α-anomer	β-anomer	α-anomer	β-anomer
Proportion	13·4%	86·6%	36·7%	63·3%
Shift of H-1[a]	5·42	5·04	5·39	5·20
$J_{1,2}$[b]	1·8	1·6	1·5	1·7
Shift of H-3[a]	[c]	4·27	4·17	4·0
$J_{3,2}$	[c]	5·6	4·7	4·7
$J_{3,4}$	[c]	12·1 4·2[d]	9·3	9·5

[a] δ-Scale (ppm); [b] in Hz; [c] These values could not be determined; [d] $J_{3,4e}$.

5 min reaction the solution was washed with 5% aqueous sodium hydrogen carbonate (100 ml) and 5% aqueous sodium thiosulfate (100 ml) and then dried. After evaporation the residue was crystallized from ether. Yield, 42 g (98%) of iodo derivative *II*, m.p. 127–128°C, $[\alpha]_D$ −88° (*c* 1·10, chloroform). Literature[8] gives m.p. 127–128°C, $[\alpha]_D$ −89° (*c* 2·02, chloroform), literature[9], m.p. 84°C, $[\alpha]_D$ −90° (*c* 1·0, chloroform).

1,6-Anhydro-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-*xylo*-hexopyranose (*III*)

Iodo derivative *II* (10 g; 23·5 mmol) in ethanol (100 ml) was hydrogenated on 10% palladium on charcoal (1 g) in the presence of sodium hydrogen carbonate (3 g) at 40–60°C, under frequent rinsing of the apparatus with hydrogen. Two products were formed during the reaction, with $R_F = 0{\cdot}35$ and $R_F = 0{\cdot}64$, TLC: benzene–acetone (10 : 1). After 48 h reaction time only the product with the lower R_F value was present in the medium. The catalyst was filtered off, the solution evaporated and the residue crystallized from an ethanol–water mixture. Yield, 6·5 g (93%) of deoxy derivative *III*, m.p. 92–93°C, $[\alpha]_D$ −36° (*c* 2·41, chloroform), mixture m.p. with an authentic sample was underpressed, literature[7] gives m.p. 93–95°C, $[\alpha]_D$ −42° (*c* 1·7, chloroform), lit.[8], m.p. 92–94°C, $[\alpha]_D$ −40° (*c* 2·0, chloroform).

1,6-Anhydro-4-deoxy-4-iodo-2-O-*p*-toluenesulfonyl-β-D-galactopyranose (*IV*)

A) The hydrogenation of iodo derivative *II* was carried out in the same manner as in the preceding case, but it was interrupted after 24 h and the reaction mixture worked up as described above. Fractional crystallization from ethanol gave a product enriched in intermediate *IV* (700 mg), which was chromatographed on a silica gel column (15 g) in benzene–acetone (20 : 1). Yield, 510 mg of iodo derivative *IV*, m.p. 157–159°C, $[\alpha]_D$ −1° (*c* 3·45, chloroform), IR spectrum: 3 580 (OH), 1 183, 1 197, 1 375 (—O—SO_2). ^{1}H NMR spectrum (60 MHz) in deuteriochloroform hexadeuteriodimethyl sulfoxide (1 : 1, TMS): 7·28–7·93 m (4 H) *p*-tolyl, 5·98 bs (1 H, OH), 5·33 t (H-1, $J_{1,2} \approx J_{1,3} \approx 1{\cdot}7$), 4·54 m (4 H, H-2, H-5, H-6_{endo}, H-6_{exo}), 3·78 m (H-3), 3·61 bs (H-4), 2·48 s (3 H, CH_3). Literature[8] gives m.p. 158–159°C, $[\alpha]_D$ −5° (*c* 3·0, chloroform).

B) A mixture of iodohydrin *II* (4·2 g, 9·9 mmol), potassium iodide (8·3 g, 50 mmol) and dimethylformamide (25 ml) was refluxed for 10 min. After cooling and dilution with water crystals separated which were filtered with suction and washed with water. From the mixture of iodo derivatives (TLC in benzene–acetone 10 : 1) 1·47 g (36%) of iodo derivative *IV* were obtained by crystallization from ethanol and chromatography on silica gel. Its properties were identical with those of the compound prepared under *A*).

Hydrogenation of iodo derivative *IV* under the same conditions as in the hydrogenation of derivative *II* gave deoxy derivative *III*, described above.

1,6-Anhydro-3-O-(N-benzylcarbamoyl)-4-deoxy-2-O-*p*-toluenesulfonyl-β-D-*xylo*-hexopyranose (*V*)

Tosylate *III* (1 g, 3·33 mmol) was dissolved in toluene and benzyl isocyanate[18] (0·5 g, 3·76 mmol) was added to it under stirring. The solution was refluxed for 5 h, TLC in ether–cyclohexane (4 : 1). After concentration an oily product (*V*) was obtained (1·5 g, 96%) which could be used without further purification for the preparation of oxazolidinone *VII*. For analytical purposes 500 mg of it were chromatographed on a silica gel column in benzene–acetone (20 : 1) and 400 mg (83%) of urethan *V* were obtained, $[\alpha]_D$ +13° (*c* 1·28, chloroform), IR spectrum: 1 730, 1 525 (—N—CO—), 1 610 (phenyl), 1 183, 1 198, 1 380 (—O—SO_2—). For $C_{21}H_{23}NO_7S$ (433·5) calculated: 58·19% C, 5·35% H, 3·23% N; found: 57·95% C, 5·33% H, 3·15% N.

3-Benzyl-(1,6-anhydro-2,3,4-trideoxy-β-D-*lyxo*-hexopyrano)-[2,3-*d*]-oxazolidin-2-one (*VII*) and 1,6 : 2,3-dianhydro-4-deoxy-β-D-*lyxo*-hexopyranose (*IX*)

Chromatographically purified urethane *V* (200 mg, 0·46 mmol) was dissolved in dimethylformamide (5 ml) and the solution was added to 30 mg of sodium hydride (60% suspension in paraffin oil, previously washed with light petroleum). The mixture formed was stirred at room temperature for 30 min; TLC: ether–cyclohexane 4 : 1. The excess of the hydride was decomposed with a few drops of ethanol and 1M-HCl was added to the mixture until the pH value dropped to pH 3. The solution was evaporated and the residue extracted with chloroform, the extract evaporated and the product (98 mg) chromatographed on a silica gel column (15 g) in a mixture of benzene–acetone (25 : 1). Yield, 11 mg (9%) of epoxide *IX*, m.p. 67–69°C, $[\alpha]_D$ −36° (*c* 0·3, water). IR spectrum identical with that of an authentic sample. Literature[7] gives m.p. 69–70°C, $[\alpha]_D$ −35° (*c* 1·0, water).

As a second fraction oxazolidinone *VII* (68 mg, 56%) was eluted from the column. After crystallization from ether, m.p. 133–134°C, $[\alpha]_D$ −108° (*c* 0·44, chloroform), IR spectrum 1 756 (C=O). For $C_{14}H_{15}NO_4$ (216·3) calculated: 64·36% C, 5·79% H, 5·36% N; found: 64·28% C, 5·76% H, 5·42% N.

When operating on a larger scale crude urethane *V* may be used and from the mixture obtained *VII* was separated by working up and crystallization from ether–light petroleum. From 12 g of tosylate *II* 4 g (38%) of crystalline oxazolidinone *VII* were so obtained.

1,6-Anhydro-2-benzylamino-2,4-dideoxy-β-D-*lyxo*-hexopyranose (*X*)

A solution of sodium hydroxide (2·5 g in 10 ml of water) was added to a solution of oxazolidinone *VII* (2 g, 7·6 mmol) in ethanol (50 ml) and the mixture was refluxed for 4 h. TLC in benzene–acetone (10 : 1), for *VII* R_F 0·64, for *X* R_F 0·42. Ethanol was then evaporated, the aqueous solution acidified with hydrochloric acid and filtered. After alkalization with solid sodium carbonate to pH 11 the mixture was extracted with three 50 ml portions of chloroform, the extract dried, concentrated and crystallized from a mixture of acetone and ether. The yield of benzylamino derivative *X* was 1·37 g (84%), m.p. 88–89°C, $[\alpha]_D$ −111° (*c* 1·17, chloroform). IR spectrum: 3 300–3 500 (OH, NH). For $C_{13}H_{17}NO_3$ (235·3) calculated: 66·36% C, 7·28% H, 5·95% N; found: 66·42% C, 7·28% H, 6·07% N.

Hydrochloride of 2-Amino-1,6-anhydro-2,4-dideoxy-β-D-*lyxo*-hexopyranose (*XI*)

Benzylamino derivative *X* (0·9 g, 3·55 mmol) was hydrogenated in ethanol (50 ml) containing azeotropic hydrochloric acid (0·65 ml) and water (0·65 ml), under catalysis with 10% palladium on charcoal (100 mg). After 4 h the reaction was terminated. TLC in chloroform–2-propanol–25% ammonia–water–ethanol (20 : 20 : 2 : 2 : 1). The catalyst was filtered off on Celite, the solution concentrated and repeatedly evaporated with water. The residue was dissolved in a minimum amount of hot water, diluted with ethanol and allowed to stand for crystallization. Yield, 600 mg (93%) of hydrochloride *XI*, decomp. at 210–220°C, $[\alpha]_D$ −64° (*c* 1·33, water.) ^{1}H NMR spectrum (200 MHz) in deuterium oxide, DSS as internal reference: 5·59 bt (H-1, $J_{1,2}$ = 1·8, $J_{1,3}$ = 1·3, $J_{1,4}$ = 0·5, $J_{1,6endo}$ = 0·5, $J_{1,6exo}$ = 0·1), 3·49 dd (H-2, $J_{2,1}$ = 1·8, $J_{2,3}$ = 5·4), 4·25 m (H-3, $J_{3,2}$ = 5·4, $J_{3,4a}$ = 4·5, $J_{3,4e}$ = 1·7, $J_{3,1}$ = 1·3, $J_{3,5}$ = 1·0), 2·29 ddt (H-4a, $J_{4a,3}$ = 4·5, $J_{4,5}$ = 4·1, $J_{4,4}$ = −15·6, $J_{4,6exo}$ = 1·6), 2·03 dt (H-4e, $J_{4,3}$ = 1·7, $J_{4,5}$ = 1·8, $J_{4,4}$ = −15·6, $J_{4,1}$ = 0·5), 4·72 m (H-5, $J_{5,4a}$ = 4·1, $J_{5,4e}$ = 1·8, $J_{5,6endo}$ = 0·9, $J_{5,6exo}$ = 5·4, $J_{5,3}$ = 1·0), 4·34 dd (H-6endo, $J_{6,5}$ = 0·9, $J_{6,6}$ = −7·3, $J_{6,1}$ = 0·2), 3·79 dt (H-6exo $J_{6,5}$ = 5·4, $J_{6,6}$ = −7·2, $J_{6,1}$ = 0·1, $J_{6,4}$ = 1·6). For $C_6H_{12}ClNO_3$ (181·6) calculated: 39·68% C, 6·66% H, 19·52% Cl, 7·71% N; found: 39·57% C, 6·72% H, 19·88% Cl, 7·46% N.

2-Acetamido-3-O-acetyl-1,6-anhydro-2,4-dideoxy-β-D-*lyxo*-hexopyranose (*XIII*)

Hydrochloride *XI* (73 mg, 0·5 mmol) was dissolved in pyridine (3 ml) and acetic anhydride (0·3 ml, 3·2 mmol) was added to it and the mixture allowed to stand at room temperature for 24 h. TLC in chloroform–methanol 10 : 1, The excess of acetic anhydride was decomposed with methanol (0·5 ml) after previous cooling of the mixture to 0°C, the solution was concentrated and the residue evaporated with toluene (2 × 5 ml) and dried in a vacuum. The product was crystallized from a mixture of acetone and ether. Yield, 60 mg (66%) of acetyl derivative *XIII*, m.p. 184°C (the substance sublimates at 175°C) $[\alpha]_D$ −31° (*c* 1·1, chloroform). IR spectrum: 3 467 (NH), (NH), 1 752 (C=O), 1 520, 1 689 (N—C=O). For $C_{10}H_{15}NO_5$ (229·2) calculated: 52·39% C, 6·60% H, 6·11% N; found: 52·47% C, 6·73% H, 5·83% N.

Hydrochloride of 2-Amino-2,4-dideoxy-D-*lyxo*-hexopyranose (*XIV*)

A solution of hydrochloride *XI* (300 mg, 1·65 mmol) in azeotropic hydrochloric acid (10 ml) was heated under a mild stream of argon at 100°C for 2 h. TLC in chloroform–2-propanol–25% ammonia–water–ethanol (20 : 20 : 2 : 2 : 1). After concentration the residue was evaporated with 5% aqueous ethanol (2 × 15 ml) and purified with charcoal in the same solvent. After evaporation and drying over potassium hydroxide 300 mg (91%) of glassy hydrochloride *XIV* were obtained, which was used directly for N-acetylation. After several months' standing the sample of the hydrochloride crystallized out. After washing with aqueous ethanol (90%) and drying it had m.p. 160–161°C, under decomposition, $[\alpha]_D$ −10·8° (3 min), −8·4° (5 min), −2·4° (15 min), −1·7° (30 min), −1·6° (60 min and 12 h) (*c* 1·66, water). Chromatography on thin-layer of cellulose (Lucefol plates, Kavalier, 20 × 20 cm) impregnated with a borate buffer[19], in pyridine–ethyl acetate–acetic acid–water (5 : 5 : 1 : 3), gave the following results: hydrochloride of 4-deoxy-D-mannosamine (*XIV*) R_F 0·39, 2-amino-2-deoxy-D-mannose (D-mannosamine) R_F 0·33, and 2-amino-2-deoxy-D-glucose (D-glucosamine) R_F 0·30. ^{1}H NMR spectrum (200 MHz) in deuterium oxide (DSS): 5·42 bs (H-1 $J_{1,2}$ = 1·8, α-anomer: 13·4%), 5·04 d (H-1, $J_{1,2}$ = 1·6, β-anomer: 86·6%), 4·27 ddd (H-3, $J_{2,3}$ = 5·6, $J_{3,4}$ = 12·1, $J_{3,4}$ = 4·2), 3·58–3·77 m (H-2, H-5, H-6, H-6'), 1·88 m (H-4), 1·40 m (H-4'). For $C_6H_{14}ClNO_4$ (199·7) calculated: 36·10% C, 7·07% H, 17·76% Cl, 7·02% N; found: 36·32% C, 7·10% H, 17·65% Cl, 7·15% N.

Hydrochloride of 2-Amino-2-deoxy-D-mannopyranose (*XV*)

^{1}H NMR spectrum, commercial preparation of the firm Fluka, in deuterium oxide (DSS): α-Anomer: 5·39 bd (H-1, $J_{1,2}$ = 1·5), 3·65 dd (H-2, $J_{2,1}$ = 1·5, $J_{2,3}$ = 4·7), 4·00 dd (H-3, $J_{3,2}$ = 4·7, $J_{3,4}$ = 9·3), 3·61 t (H-4, $J_{4,3} \approx J_{4,5} \approx 9{\cdot}6$), ≈ 3·97 m (H-5), 3·86 m (H-6, $J_{6,6'}$ = 12·2, $J_{6,5}$ = 1·7), 3·82 m (H-6', $J_{6',6}$ = 12·2, $J_{6',5}$ = 5·9). β-Anomer: 5·20 d (H-1, $J_{1,2}$ = 1·7), 3·72 dd (H-2, $J_{2,1}$ = 1·7, $J_{2,3}$ = 4·7), 4·17 dd (H-3, $J_{3,2}$ = 4·7, $J_{3,4}$ = 9·5), 3·54 (H-4, $J_{4,3} \approx J_{4,5} \approx 9{\cdot}5$), 3·47 (H-5, $J_{5,6}$ = 2·2, $J_{5,6'}$ = 5·2, $J_{5,4}$ = 9·9), 3·91 dd (H-6, $J_{6,6'}$ = 12·3, $J_{6,5}$ = 2·2), 3·76 dd (H-6', $J_{6,6}$ = 12·3 $J_{6',5}$ = 5·2).

The integration curve of the signals corresponding to the protons H-1 indicated the ratio of the anomers α : β = 36·7 : 63·3.

2-Acetamido-2,4-dideoxy-D-*lyxo*-hexopyranose (*XVI*)

An aqueous solution of hydrochloride *XIV* (200 mg, 1 mmol in 1 ml) was added to a mixture of Dowex 1 × 8 (100–200 mesh, 15 ml) in carbonate form and water (5 ml) cooled at 2°C, and then acetic anhydride (0·7 ml, 7 mmol) was added to it dropwise under stirring and cooling. The mixture was stirred for 90 min. TLC in ethyl acetate–2-propanol–pyridine–water (7 : 3 : 1 : 2).

The ion exchanger was filtered off, washed with water and the combined filtrates were introduced into a column with Amberlite IR 120 (15 ml) in H^+ form, which was then washed with water. The fractions containing the N-acetate *XVI* were combined and concentrated. After drying 175 mg (85%) of a glassy product *XVI* were obtained, $[\alpha]_0$ +6° (10 min) → +4·5° (equilibrium after 12 h, *c* 1·32, water). The purity of the sample was checked by gas chromatography of trimethylsilyl derivatives (T_k = 206°C, v_{N_2} = 12 ml min^{-1}, column length 2 m, diameter 2·5 mm, packed with Gas-Chrom Q coated with 5% OV 210: t_1 = 1·85 min, t_2 = 2·2 min, ratio $h_1 : h_2$ = = 1 : 3·2). For $C_8H_{15}NO_5$ (205·2) calculated: 46·82% C, 7·37% H, 6·83% M; found: 47·01% C, 7·40% H, 6·52% N.

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