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Synthesis of Modified Aldonic Acids and Studies of Their Substrate Efficiency for Dihydroxy Acid Dehydratase (DHAD)*

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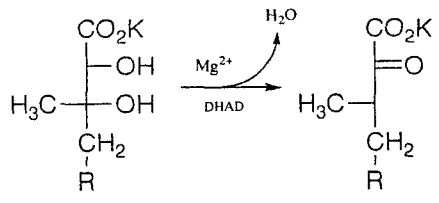
Modified aldopentonic and aldohexonic acids were synthesized in order to study the electronic requirements for a successful enzymatic conversion into their corresponding 2-keto 3-deoxy analogues by dihydroxy acid dehydratase (DHAD), an enzyme from the biosynthetic pathway of branched chain amino acids. Analytical tests with the novel artificial substrates (18)–(21) and (27) provided evidence that the amount of conversion could be enhanced by replacement of the hydroxy group at C4 of L-arabinonic acid (21) with less electron-withdrawing, ambivalent or electron-donating substituents. Modified aldohexonic acids were no substrates for DHAD, perhaps due to less perfect binding to the active site presumably for steric reasons. For 4-deoxy-L-*threo*-pentonic acid (18) the enzymatic conversion into 3,4-dideoxy-2-ketopentonic acid (29) by DHAD could be achieved on a preparative scale.

Introduction

DHAD catalyses the dehydration of its two natural substrates (2*R*)-2,3-dihydroxyisovaleric acid (1) and (2*R*,3*R*)-2,3-dihydroxy-3-methylvaleric acid (2) (shown in Scheme 1 as their potassium salts) to their corresponding 2-keto acid analogues [2-oxoisovaleric acid (3) and (3*S*)-3-methyl-2-oxovaleric acid (4)]. The enzyme is involved in the biosynthesis of the branched chain amino acids valine, isoleucine and leucine in higher plants, bacteria, algae and yeast.[1]



(1) R = H
(2) R = CH_3
(3) R = H
(4) R = CH_3

Scheme 1

For substrate recognition by DHAD a 2,3-dihydroxy acid with (2*R*) configuration is required. In the case of the diastereomeric 2,3-dihydroxy-3-methylvaleric acids both the natural (2*R*,3*R*) isomer (2) and the artificial (2*R*,3*S*) isomer are accepted as substrates for DHAD. The reaction rate for the artificial *threo* configured dihydroxy acid is found to be even higher than for the natural *erythro* isomer.[2]

The catalytic mechanism of DHAD has been partly investigated. By labelling experiments Arfin *et al.*[3] proved the formation of an enol intermediate in the course of the reaction. Later results gave evidence for a stereoselective rearrangement of the enzyme-bound enol towards the 2-keto acid. Flint *et al.*[4] observed an iron–sulfur cluster located at the active site of the enzyme and postulated that it is involved in the catalytic process like a Lewis acid in the initial cleavage of the hydroxy group at C3. Pirrung *et al.*[5] confirmed this postulated cationic-like intermediate, and also presented evidence for an enzyme-bound enol by studying intermediate analogue inhibitors. The principal results of all these investigations are summarized in Scheme 2 as a postulated catalytic mechanism.

Only a few studies with regard to substrate modifications have been carried out. Armstrong *et al.*[6] tested substrates with longer aliphatic chains at C3, and observed low activity for a dihydroxy acid containing a seven-carbon chain. Pirrung *et al.*[5] studied the influence of $+I$ (such as cyclopropyl) and $-I$ (e.g. fluoromethyl

* This paper is dedicated to Stephen J. Angyal an ideal as friend and scientist.

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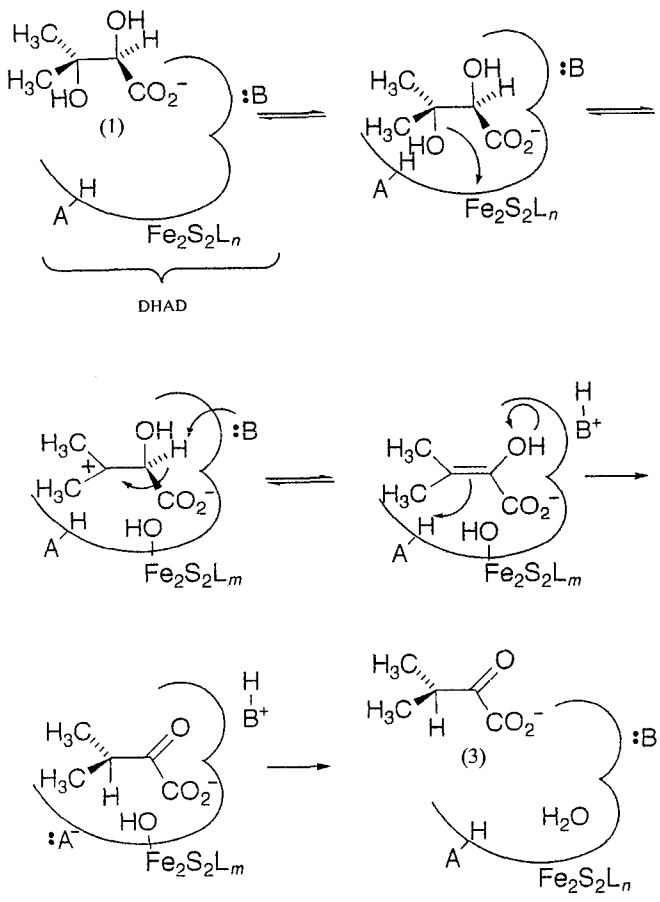


Scheme 2

and trifluoromethyl) substituents at C3, and observed that turnover rates were significantly diminished by replacement of one methyl group by a fluoromethyl or a trifluoromethyl group. However, the replacement by a cyclopropyl group led to no significant change in reaction yields compared to the natural substrate (1). In a previous investigation[7] it was shown that the broad application of DHAD in the chemoenzymatic synthesis of 2-keto 3-deoxy aldonic acids was restricted by two factors. First, aldonic acids with more than six carbon atoms were not accepted due to steric requirements which prohibited an effective binding to the active site. Second, studies of substrate analogous 3-deoxy aldonic acids as inhibitors for DHAD showed a correlation between decreasing binding capacity and increasing chain length. A notable inhibition effect could even be detected for 3-deoxy-D-*xylo*-hexonic acid.

The low reaction rates of aldopentonic and aldohexonic acids in incubations with DHAD were postulated to be due to the destabilization effect of the cationic-like intermediate, initially formed in the course of the enzymatic reaction, by the electron-withdrawing character of the hydroxy group at C4. This assumption was supported by the results of kinetic investigations for D-erythronate and L-threonate. Both showed K_M values in the range of magnitude of the natural substrate (1) but reaction rates of only 2 and 30%, respectively, compared to (1). Further, there was a contradiction between low enzymatic conversion for L-arabinonic acid (21) of about 2% and quite a strong inhibition of DHAD by its 3-deoxy analogue ($K_i/K_M(1) = 9 \cdot 2$)[7]. Owing to these observations it was decided to evaluate and study in detail the electronic restrictions for a successful application of DHAD required for the conversion of aldonic acids. The objective was to work out guidelines for substrate modifications which would permit the use of DHAD for chemoenzymatic syntheses on a preparative scale.

Chemical Synthesis of Modified Aldonic Acids

For the investigations four aldopentonic acids (18)–(21) with different electronic character at C4 were chosen. Deoxygenation at C4 as in (18) should give a weak electron-donating effect, which would lead to stabilization of the cationic-like intermediate at C3. Methylation of the hydroxy group at C4 as in (19) should cause an electron-donating effect, but at the same time it could give rise to further steric hindrance and reduce an effective binding to the enzyme. Replacement of the hydroxy group at C4 of (21) by an amino group to give the amino aldonic acid (20) with D-*xylo* configuration should lead to a slightly diminished electon-withdrawing character of C4 compared to (21). However, other interactions due to the basicity of the amino group with acidic centres of the protein as well as a significant change of the substrate structure in solution by intramolecular acid base interactions should be taken into consideration.

To our knowledge aldopentonic acids with substituents other than a hydroxy group at C4 had not been synthesized before and no common strategy for the introduction of new substituents at this position was available from literature. Recently, well established techniques for the introduction of an azido group or deoxygenation at C4 of the corresponding aldoses (5) and (22) were published.[8] Starting from L-arabinose (5) the partially benzoylated glycoside (6) could be obtained in 66% yield according to the procedure of Jones *et al.*[9] Following the synthetic approach of Klaffke *et al.*[8] the D-*xylo* iodide (8) was synthesized by initial treatment with triflic anhydride (Tf_2O) and subsequent nucleophilic displacement with sodium iodide in 98% yield. Reductive deoxygenation gave (9), and consecutive cleavage of the benzoyl (Bz) and benzyl (Bn) protecting groups yielded 4-deoxyarabinose (14) in 71% overall yield. Finally, the oxidation of (14) with iodine in alkali/methanol by the method of Moore *et al.*[10] enabled the synthesis of the desired 4-deoxy acid (18) in 61% yield (see Scheme 3).

For protection of the hydroxy group at C4 in (6) as a methyl ether, the acidic methylation with methyl trichloroacetimidate was used.[11] A similar approach for acidic benzylation with benzyl trichloroacetimidate was published recently by Bundle *et al.*[12] Treatment of

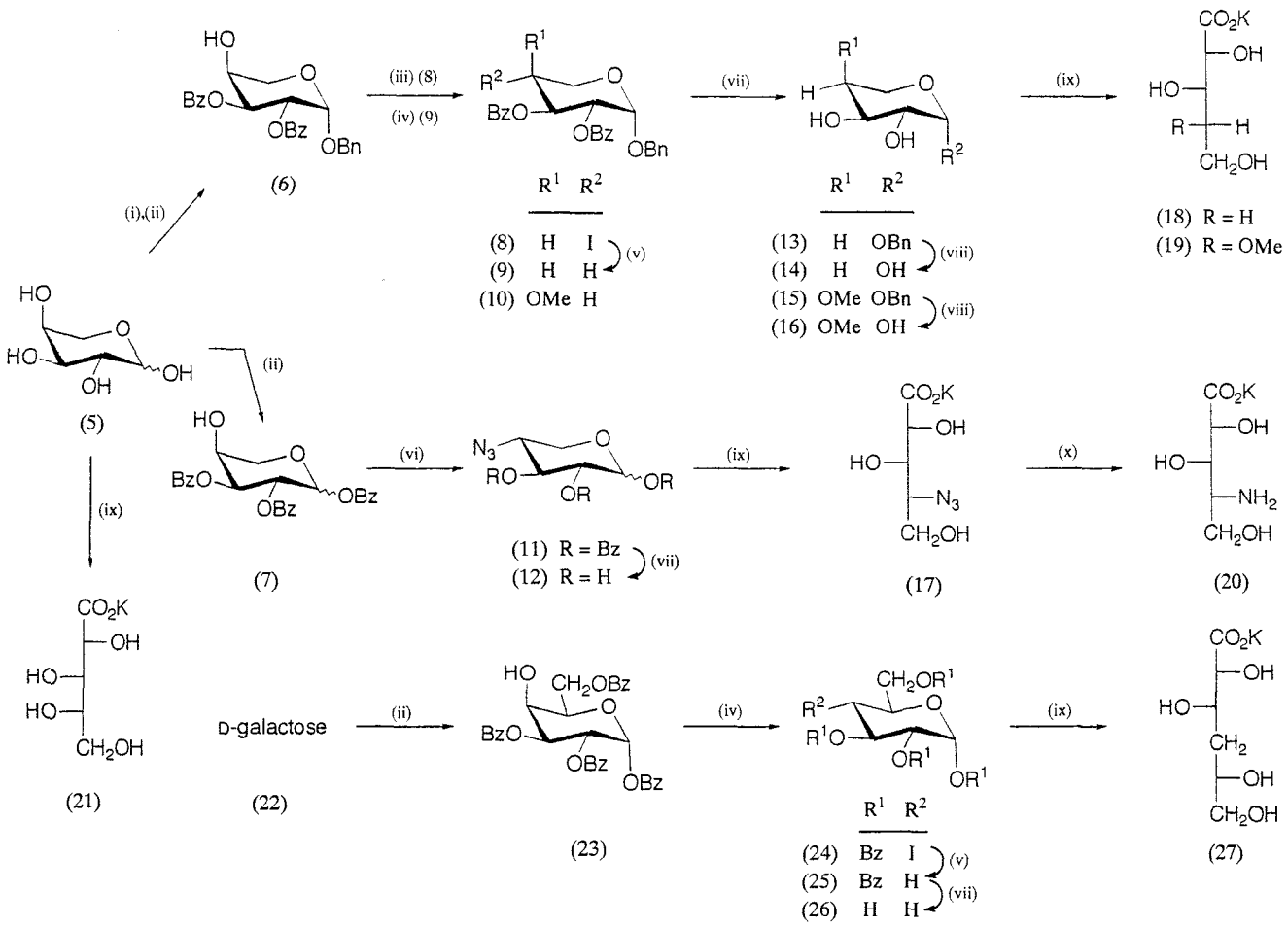
(6) with a large excess of methyl trichloroacetimidate and catalytic amounts of trifluoroacetic acid (pH ≈ 2) for 7 days led to the formation of about 50% (from n.m.r.) of the desired product (10). In addition 20% of starting material (6) and 25% of another methylated compound, identified after debenzoylation as benzyl 5-*O*-methyl-β-L-arabinofuranoside, were isolated. Separation was favourably done of the crude mixture after the debenzoylation procedure and this allowed the isolation of (15) in 40% yield based on (6). Cleavage of the glycoside gave (16) quantitatively, and oxidation as described for (18) led to the isolation of the 4-methoxy acid (19) in 70% overall yield.

Starting with compound (6) an azido group could be introduced to give benzyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-D-xylopyranoside. Simultaneous hydrogenation of the azide liberated the amine which following hydrogenolysis of the anomeric benzyl group gave rise to the formation of the corresponding aminocyclitol. Therefore, a selective benzoylation of L-arabinose (5) as described by Williams *et al.*[13] and Lindhorst[14] was carried out. The desired product (7) had to be separated by laborious chromatography on silica gel from several dibenzoylated pyranoses and tribenzoylated furanoses to give pure (7) as a mixture of anomers in only 23% yield. The azido group was introduced by analogy to the formation of the iodide (8) by treatment of (7) with triflic anhydride and nucleophilic displacement with sodium azide in 70% yield. Deprotection of (11) and oxidation as described above led to the 4-azido aldonic acid (17) in 66% overall yield. Finally the amino group was generated by catalytic hydrogenation to yield 86% of the 4-amino aldonic acid (20) with D-*xylo* configuration.

Due to the convincing results in the enzymatic tests for (18), the synthesis of the corresponding 4-deoxy aldohexonic acid (27) was started. Compound (25) was obtained as described by Klaffke *et al.*[8] in 11% overall yield starting with D-galactose (22). After cleavage of the benzoyl esters and oxidation with iodine in alkaline methanol 4-deoxy-D-*xylo*-hexonic acid (27) was obtained in 54% overall yield.

Enzymatic Tests of the Unnatural Substrates

The homogeneity of the chemically synthesized new compounds (18)–(21) and (27) was proved by n.m.r.,



Scheme 3. Reagents: (i) BnOH, HCl(g); (ii) BzCl, pyridine, CH_2Cl_2, −40°C; (iii) Tf_2O, pyridine, CH_2Cl_2, −20°C; then NaI, dimethylformamide; (iv) MeO-C(CCl_3)=NH, TfOH (pH 2); (v) H_2, Pd–C, EtOH, $CHCl_3$, $NaHCO_3$(aq); (vi) Tf_2O, pyridine, CH_2Cl_2, −20°C; then NaN_3, dimethylformamide; (vii) NaOMe, MeOH; (viii) H_2 (30 bar), Pd–C, MeOH; (ix) I_2, KOH, MeOH (40°C); (x) H_2, Pd–C, H_2O.

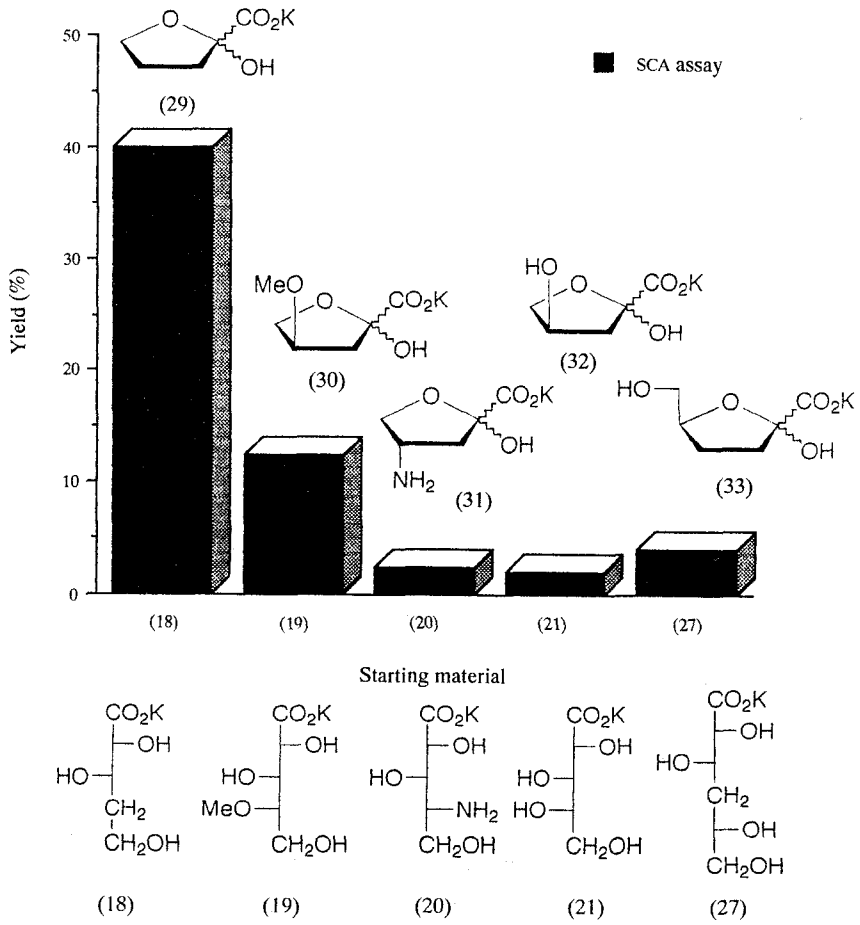
elemental analysis and h.p.l.c. DHAD was purified from spinach leaves as described previously.[7] A partially purified protein solution containing 0·66 U/mg protein (1 U = formation of 1 μmol product (3) per min) was obtained, and this corresponded to a 22-fold enrichment of the crude solution. The enzyme solution was checked for undesired side activities by incubation of the natural substrate (1) for 7 days at 35°C. Also the chemically synthesized 3-deoxy-2-keto-D-*threo*-hexonic acid (28),[15] as a representative product, was incubated similarly, and in both experiments no significant change of products was observed. The quantititative determination was performed by a colorimetric assay based on the reaction of semicarbazide with the enzymatically formed 2-keto acid to give the corresponding semicarbazone (SCA assay).[7,16] Photometry at 250 nm allowed the quantitative evaluation of the absorbance based on the calibration plots of products (3) and (28).

For analytical investigation of the substrate capacity 10 mM solutions of the artificial substrates (18)–(21) and (27) together with 20 mU of purified DHAD were incubated. The product formation was followed by the SCA assay and showed 92% conversion for the natural substrate (1) after an incubation for 3 days at 35°C. The results for the aldonic acids (18)–(21) and (27) are summarized in Scheme 4.

As shown in Scheme 4 the yields for the aldopentonic acids (18)–(21) decreased with increasing electron-withdrawing character for the new substituents at C 4. This relationship supported the assumption that an electron-rich substituent at C 4 would stabilize the cationic intermediate at C 3 which is initially formed in the course of the enzymatic reaction, and vice versa. The convincing results for (18) encouraged us to test the corresponding aldohexonic acid (27) as a substrate for DHAD. However, the chain elongation led to a significant drop of the conversion rates probably by reduced binding to the active site. A corresponding phenomenon was shown in previous inhibition studies.[7] The inhibitor capacity of 3-deoxy-L-*threo*-pentonic acid ($K_i/K_M(1) = 9 \cdot 2$) was found to be about twofold higher than for 3-deoxy-D-*arabino*-hexonic acid ($K_i/K_M(1) = 14 \cdot 2$). On the basis of the previous and these results it may be concluded that a compound designed as a favourable artificial substrate for DHAD should be a (2*R*,3*S*) 2,3-dihydroxy acid containing electron-donating groups at C 3 and/or C 4, and a carbon chain not exceeding five atoms. Therefore, an aldonic acid containing a chain branch at C 3 or C 4 by introduction of, e.g., a methyl group should be considered to be one of the best artificial substrates.

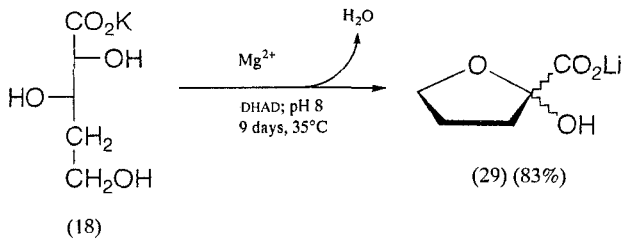
Chemoenzymatic Synthesis of (29)

In addition to analytical investigations the use of DHAD for a chemoenzymatic synthesis on a preparative



Scheme 4

scale was of interest. On the basis of the previously reported successful application of DHAD for a preparative conversion of L-threonate into the corresponding lactonized 2-keto 3-deoxy analogue,[7] compound (18) was chosen as another example for a preparative-scale synthesis catalysed by DHAD. Incubation of 0·1 mmol of (18) together with 1·5 U of DHAD in TRIS buffer at 35°C for 3 days gave 40% of product as detected by the SCA assay. Further addition of enzyme (each 1·5 U) led to 79% yield after 6 days and 99% yield after 9 days. Removal of proteins by membrane filtration and treatment with cation-exchange resin followed by addition of lithium hydroxide to pH 7·5 yielded crude (29). Removal of inorganic salts by chromatography on Sephadex G 10 allowed the isolation of pure 3,4-dideoxy-2-ketopentonic acid (29) in 83% yield (see Scheme 5).



Scheme 5

This example demonstrated the potential of DHAD for preparative applications. Apparently, a significant interaction between product and enzyme does not take place either by equilibrium formation between substrates and products or by inhibition of the enzyme by large amounts of product. This advantage can be used for incubations with quantitative consumption of starting material by using the enzyme in excess, which is easily accessible from spinach leaves in larger quantities.

However, due to the observed rather strict substrate requirements, the application of DHAD for general preparative-scale synthesis of 2-keto 3-deoxy aldonic acids is limited.

Experimental

General

N.m.r. spectra were recorded on a Bruker AMX 400 instrument: ^1H (400 MHz); ^{13}C (100·6 MHz). As internal standards, $SiMe_4$ for samples in $CDCl_3$ and CH_3CN for samples in D_2O were used. Chemical shifts are given in ppm downfield from $SiMe_4$, and J values in Hz. Melting points were taken by using an Olympus polarizing microscope and are uncorrected. T.l.c. was carried on silica gel (60 F_{254} Merck) on aluminium foil. Preparative column chromatography was performed on silica gel (60, 230–400 mesh, 0·040–0·063 mm, Merck) by using the flash technique. For desalting Sephadex G-10 was used. Optical rotations were measured at *c.* 20°C by using a Perkin–Elmer 241 polarimeter and 1-dm cuvette. All chemicals were purchased from Merck, Aldrich, Fluka and Sigma.

Enzyme Purification

Purification of DHAD from fresh spinach leaves was carried out as previously described in ref. 7.

Semicarbazide Assay

Samples of the protein solutions (10–200 μl) were mixed with a solution of racemic substrate (1)+enantiomer (100 μl, 80 mM) in TRIS buffer (100 mM TRIS.HCl, pH 8, 20 mM $MgCl_2$) or the artifical substrates (18)–(21) and (27) at a concentration of 40 mmol l^{-1} and broght to a final volume of 400 μl by addition of TRIS buffer. After 30 min incubation at 35°C the reaction was stopped by the addition of 2 N aq. HCl (100 μl). Than a solution (300 μl) containing semicarbazide hydrochloride (1·0 g) and sodium acetate (1·5 g) in doubly distilled water (100 ml) was added. The solution was kept for 15 min at 30°C, diluted with doubly distilled water (500 μl), degassed by ultrasonication and centrifuged for 5 min. The absorbance of the obtained solution was measured at 250 nm and evaluated by means of a calibration plot for the commercial product (3) or the chemically synthesized[15] compound (28).

Benzyl β-L-Arabinopyranoside (6)

Compound (6) was prepared as described by Jones *et al.*[9] in 66% overall yield.

1,2,3-Tri-O-benzoyl-L-arabinopyranose (7)

Compound (5) (5·0 g, 33 mmol) was dissolved in hot anhydrous pyridine (100 ml). The solution was cooled to −40°C and treated dropwise over 1 h with benzoyl chloride (11·6 ml, 100 mmol). After 4 h at −40°C, CH_2Cl_2 (100 ml) was added. The organic layer was washed several times with 2 N HCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and evaporated under vacuum. After several azeotropic distillations, the crude mixture was subjected to column chromatography (toluene/ethyl acetate 30 : 1 followed by 20 : 1 and 10 : 1) to give (7) (3·17 g) in 23% yield. The analytical data are in accord with ref. 13.

Benzyl 2,3-Di-O-benzoyl-4-deoxy-4-iodo-α-D-xylopyranoside (8)

Compound (6) (5·0 g, 11·15 mmol) was dissolved in anhydrous CH_2Cl_2 (50 ml) and anhydrous pyridine (7 ml), and treated dropwise under a nitrogen cover with a solution of triflic anhydride (5 ml, 30·5 mmol) in anhydrous CH_2Cl_2 (30 ml) at −20°C. After 2 h, the mixture was poured into ice water, and extracted twice with CH_2Cl_2. The organic layer was dried over $MgSO_4$, evaporated under vacuum at a maximum of 30°C and subjected to several azeotropic distillations with toluene. The crude triflate was dissolved in anhydrous dimethylformamide (50 ml), and sodium iodide (5·0 g, 33 mmol) was added. The mixture was stirred overnight at room temperature and diluted with CH_2Cl_2 (100 ml). The orgic layer was washed twice with a 10% aqueous solution of sodium thiosulfate, dried over $MgSO_4$ and evaporated under vacuum. The crude product was subjected to column chromatography (toluene/ethyl acetate 30 : 1) to give (8) (6·12 g, 98%) as a colourless syrup; $[\alpha]_D$ +101° (*c*, 1·0 in $CHCl_3$). δ_H ($CDCl_3$) 7·94, 7·48, 7·35, 4H, 2H, 4H, m, m, m, Bz; 7·25–7·15, 5H, m, Bn; 6·04, 1H, ddd, $J_{2,3}$ 9·5, $J_{3,4}$ 6·0, $J_{3,4ax}$ 3·5, H 3; 5·32, 1H, d, $J_{1,2}$ 3·5, H 1; 5·01, 1H, dd, H 2; 4·80, 4·52, 1H, 1H, d, d, PhC**H**$_2$; 4·23, 1H, ddd, $J_{4,5ax}$ 7·5, $J_{5ax,5eq}$ 12·0, H 5*ax*; 4·19, 1H, dd, $J_{4,5eq}$ 5·0, H 5*eq*; 3·95, 1H, m, H 4. δ_C ($CDCl_3$) 164·9, 164·6, Bz; 136·2–127·0, Bz, Bn; 95·1, C 1; 72·2, 72·0, C 2, C 3; 69·1, Ph**C**H$_2$; 63·8, C 5; 21·8, C 4.

Benzyl 2,3-Di-O-benzoyl-4-deoxy-β-L-threo-pentopyranoside (9)

Compound (8) (6·0 g, 10·75 mmol) was dissolved in ethanol (25 ml), $CHCl_3$ (25 ml) and saturated aqueous $NaHCO_3$. Palladium on carbon (10%, 500 mg) was added and the mixture was

stirred for 18 h in a hydrogen atmosphere at room temperature. The catalyst was filtered off and CH_2Cl_2 (200 ml) was added. The organic layer was washed with sodium thiosulfate and water, dried over $MgSO_4$ and evaporated under vacuum to give pure (9) (3·9 g, 87%) as a colourless syrup; $[\alpha]_D$ +144·2° (*c*, 1·0 in $CHCl_3$). δ_H ($CDCl_3$) 7·94, 7·48, 7·35, 4H, 2H, 4H, m, m, m, Bz; 7·30–7·15, 5H, m, Bn; 5·73, 1H, ddd, $J_{2,3}$ 10·2, $J_{3,+eq}$ 5·0, $J_{3,4ax}$ 11·2, H3; 5·27, 1H, dd, $J_{1,2}$ 3·5, H2; 5·22, 1H, d, H1; 4·78, 4·25, 1H, 1H, d, d, PhC**H**$_2$; 4·02, 1H, ddd ≈ dt, $J_{4ax,5ax}$ 13·0, $J_{4eq,5ax}$ 2·5, $J_{5ax,5eq}$ 13·0, H5*ax*; 3·74, 1H, ddd, $J_{4ax,5eq}$ 5·0, $J_{4eq,5eq}$ 3·0, H5*eq*; 2·31, 1H, m, $J_{4ax,4eq}$ 13·0, H4*eq*; 1·96, 1H, dddd, H4*ax*. δ_C ($CDCl_3$) 165·4, 165·3, Bz; 136·8–126·9, Bz, Bn; 95·6, C1; 72·1, C2; 68·7, Ph**C**H$_2$; 68·3, C3; 57·1, C5; 30·6, C4.

*4-Azido-1,2,3-tri-*O*-benzoyl-4-deoxy-*D*-xylopyranose (11)*

Compound (7) (5·1 g, 11 mmol) was dissolved in anhydrous CH_2Cl_2 (50 ml) and pyridine (7 ml), and treated with triflic anhydride (5 ml, 30·5 mmol) as described for (8). The crude triflate was dissolved in anhydrous dimethylformamide (50 ml), and sodium azide (2·15, 33 mmol) was added. After 18 h at room temperature, the mixture was diluted with CH_2Cl_2 and washed twice with water. The organic layer was dried over $MgSO_4$ and evaporated under vacuum. The crude product was subjected to column chromatography (toluene/ethyl acetate 20:1) to give pure (11) (3·76 g, 70%) as a white *solid* in an $\alpha:\beta$ ratio of 1:1·2 (Found: C, 64·0; H, 4·5; N, 8·1. $C_{26}H_{21}N_3O_7$ requires C, 64·0; H, 4·4; N, 8·6%). α-Anomer: δ_H ($CDCl_3$) 8·20–7·90, 7·60–7·10, 6H, 9H, m, m, Bz; 6·72, 1H, d, $J_{1,2}$ 3·5, H1; 6·02, 1H, dd ≈ t, $J_{2,3}$ 9·5, $J_{3,4}$ 9·5, H3; 5·50, 1H, dd, H2; 4·05, 2H, m, H4, H5; 3·89, 1H, dd, $J_{4,5'}$ 7·0, $J_{5,5'}$ 12·5, H5′). β-Anomer: δ_H 8·20–7·90, 7·60–7·10, 6H, 9H, m, m, Bz; 6·17, 1H, d, $J_{1,2}$ 6·0, H1; 5·65, 2H, m, H2, H3; 4·32, 1H, dd, $J_{4,5eq}$ 4·5, $J_{5eq,5ax}$ 12·0, H5*eq*; 3·96, 1H, ddd ≈ dt, $J_{3,4}$ 8·0, $J_{4,5ax}$ 8·5, H4; 3·75, 1H, dd, H5*ax*. α- and β-Anomer: δ_C ($CDCl_3$) 165·8–164·4, Bz; 134·0–128·3, Bz; 91·9, 90·3, C1α,β; 71·2, 71·1, 70·5, 70·2, C2α,β, C3α,β; 63·6, C5α,β; 59·6, 59·5, C4α,β.

*Benzyl 4-Deoxy-β-*L*-threo-pentopyranoside (13)*

Compound (9) (3·8 g, 8·8 mmol) was dissolved in anhydrous methanol (50 ml), and treated with a freshly prepared *c.* 0·1 M solution of sodium methanolate in anhydrous methanol (0·5 ml). After 18 h at room temperature the reaction was stopped by addition of 1 drop of acetic acid, and the solvent was removed. Column chromatography (toluene/acetone 4:1) afforded (13) (1·7 g, 82%) as a white *solid*; m.p. 119°C (dec.); $[\alpha]_D$ +187·1° (*c*, 1·0 in $CHCl_3$) (Found: C, 64·3; H, 7·2. $C_{12}H_{16}O_4$ requires C, 64·3; H, 7·2%). δ_H ($CDCl_3$) 7·37–7·26, 5H, m, Bn; 4·95, 1H, d, $J_{1,2}$ 3·5, H1; 4·74, 4·47, 1H, 1H, d, d, PhC**H**$_2$; 3·83, 1H, m, H3; 3·77, 1H, ddd ≈ dt, $J_{4ax,5ax}$ 13·0, $J_{4eq,5ax}$ 2·5, $J_{5ax,5eq}$ 11·7, H5*ax*; 3·65, 1H, ddd, $J_{4ax,5eq}$ 5·0, $J_{4eq,5eq}$ 2·0, H5*eq*; 3·38, 1H, dd, $J_{2,3}$ 9·2, H2; 2·59, 2·21, 1H, 1H, s, s, 20H; 1·94, 1H, dddd, $J_{3,4eq}$ 5·1, $J_{4ax,4eq}$ 13·0, H4*eq*; 1·67, 1H, $J_{3,4ax}$ 11·2, dddd, H4*ax*. δ_C ($CDCl_3$) 14·1, Bn; 128·2–127·4, Bn; 97·6, C1; 73·9, C2; 68·9, PhC**H**$_2$; 68·6, C3; 58·0, C5; 31·8, C4.

*4-Deoxy-*L*-threo-pentopyranose (14)*

Compound (13) (1·6 g, 7·1 mmol) was dissolved in methanol (30 ml), and palladium on carbon (10%, 250 mg) was added. The mixture was kept for 30 h in an atmosphere of hydrogen at 30 bar. The catalyst was filtered off and the solvent was evaporated under vacuum to give pure (14) (950 mg, quantitative yield) as a white *solid* in an $\alpha:\beta$ ratio of 1:2·4 (Found: C, 44·7; H, 7·6. $C_5H_{10}O_4$ requires C, 44·8; H, 7·5%). α-Anomer: δ_H (D_2O/CH_3CN) 4·53, 1H, d, $J_{1,2}$ 7·5, H1; 3·99–3·91, 1H, m, H5*eq*; 3·72, 1H, ddd, $J_{2,3}$ 9·0, $J_{3,4ax}$ 11·0, $J_{3,4eq}$ 5·0, H3; 3·57, 1H, ddd, $J_{4ax,5ax}$ 12·0, $J_{4eq,5ax}$ 12·0, $J_{5ax,5eq}$ 12·0, H5*ax*; 3·18, 1H, dd, H2; 2·01, 1H, m, H4*eq*; 1·67, 1H, m, H4*ax*. δ_C (D_2O/CH_3CN) 95·8, C1; 75·2, C2; 70·0, C3; 60·9, C5; 31·8, C4. β-Anomer: δ_H (D_2O/CH_3CN) 5·24, 1H, d, $J_{1,2}$ 3·5, H1; 3·99–3·91, 2H, m, H3, H5; 3·76–3·67, 1H, m, H5′; 3·50, 1H, dd, $J_{2,3}$ 9·0, H2; 2·01, 1H, m, H4; 1·67, 1H, m, H4′. δ_C (D_2O/CH_3CN) 91·6, C1; 72·1, C2; 66·3. C3; 57·4, C5; 31·1, C4.

*Benzyl 4-O-Methyl-β-*L*-arabinopyranoside (15)*

Compound (6) (3·55 g, 7.92 mmol) was dissolved in CH_2Cl_2 (10 ml), and light petroleum (10 ml) and methyl trichloroacetimidate (3 g, 17 mmol) were added. The pH was kept at 2 by addition of trifluoromethanesulfonic acid (*c.* 0·3 ml), and further methyl trichloroacetimidate (3 g, 17 mmol) was added after 2 and 4 days. After 7 days the precipitate which had formed was removed by filtration and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$, dried over $MgSO_4$ and evaporated under vacuum. The crude mixture was subjected to column chromatography (toluene/ethyl acetate 10:1) to yield the partially purified product (10). For debenzoylation the crude (10) was treated as described for the preparation of (13). Column chromatography (toluene/acetone 4:1) afforded (15) (813 mg, 40%) as a white solid; m.p. 48°C; $[\alpha]_D$ +195·7° (*c*, 0·52 in $CHCl_3$). A correct elemental analysis could not be obtained for this highly hygroscopic product. δ_H (D_2O/CH_3CN) 7·49–7·39, 5H, m, Bn; 5·02, 1H, d, $J_{1,2}$ 4·0, H1; 4·75, 4·60, 1H, 1H, d, d, PhC**H**$_2$; 3·92, 1H, dd, $J_{2,3}$ 10·5, $J_{3,4}$ 3·5, H3; 3·86, 1H, dd, $J_{4,5}$ 2·5, $J_{5,5'}$ 12·5, H5; 3·78, 1H, dd, H2; 3·77, 1H, dd, $J_{4,5'}$ 1·0, H5′; 3.63, 1H, dd, H4; 3·43, 3H, s, OMe. δ_C ($CDCl_3$) 136·5, Bn; 127·9–127·4, Bn; 97·5, C1; 77·6, C2; 69·7, C3, C4; 69·2, Ph**C**H$_2$; 58·2, C5; 56·7, OMe.

*4-O-Methyl-*L*-arabinopyranose (16)*

Compound (15) (1·31 g, 5·2 mmol) was hydrogenated as described for (14). After remocal of the solvent, compound (16) (813 mg) was obtained in 96% yield as a colourless *syrup* in an $\alpha:\beta$ ratio of 2:1 (Found: C, 43·9; H, 7·5. $C_6H_{12}O_5$ requires C, 43·9; H, 7·4%). α-Anomer: δ_H (D_2O/CH_3CN) 4·50, 1H, d, $J_{1,2}$ 7·6, H1; 4·11, 1H, dd, $J_{4,5}$ 2·0, $J_{5,5'}$ 13·5, H5; 3·69, 1H, dd, $J_{2,3}$ 9·5, $J_{3,4}$ 3·5, H3; 3·59, 1H, m, H4; 3·53, 1H, d, H5′, 3·45, 1H, dd, H2; 3·43, 3H, s, OMe. δ_C (D_2O/CH_3CN) 95·8, C1; 77·3, C2; 71·3, C3; 67·4, C4; 61·4, C5; 56·2, OMe. β-Anomer: δ_H (D_2O/CH_3CN) 5·22, 1H, d, $J_{1,2}$ 3·6, H1; 3·92, 1H, dd, $J_{2,3}$ 10·0, $J_{3,4}$ 3·6, H3; 3·90, 1H, d(br), $J_{5,5'}$ 13, H5; 3·84, 1H, dd, $J_{4,5'}$ 2·5, H5′; 3.75. 1H, dd, H2; 3.64, 1H, m, H4; 3·43, 3H, s, OMe. δ_C (D_2O/CH_3CN) 91·6, C1; 77·6, C2; 71·3, C3; 67·9, C4; 56·6, C5; 56·2, OMe.

*Potassium Salt of 4-Azido-4-deoxy-*D*-xylonic Acid Monohydrate (17)*

Compound (11) (1·305 g, 2·68 mmol) was debenzoylated as described for the preparation of (13). The crude product (12) was subjected to the oxidation procedure according to ref. 10 without further purification. To the alkaline methanolic solution of (12), iodine (1·6 g, 6·4 mmol) and a solution of potassium hydroxide (1·43 g, 12·8 mmol) in methanol (20 ml) were added dropwise over 90 min. After heating for an additional 30 min to 40°C product (17) crystallized at 4°C overnight. The solid *product* was filtered off and washed several times with cold methanol to give pure (17) (440 mg) in 66% yield; m.p. 130°C (dec.); $[\alpha]_D$ +13·6°C (*c*, 0·76 in H_2O) (Found: C, 24·0; H, 4·0; N, 10·6. $C_5H_{10}KN_3O_6$ requires C, 24·3; H, 4·1; N. 17·0%). δ_H (D_2O/CH_3CN) 4·12, 1H, d, $J_{2,3}$ 3·0, H2; 4·02, 1H, dd, $J_{3,4}$ 7·0, H3; 3·94, 1H, ddd ≈ dt, $J_{4,5}$ 6·5, $J_{4,5'}$ 9.5, H4; 3·78, 2H, m, H5, H5′. δC (D_2O/CH_3CN) 177·0, C1; 71·2, 70·9, C2, C3; 64·5, C4; 60·1, C5.

*Potassium Salt of 4-Deoxy-*L*-threo-pentonic Acid Monohydrate (18)*

Compound (14) (652 mg, 4·9 mmol) was oxidized as described for (17). After crystallization at room temperature, pure (18) (623 mg, 61%) was obtained as a white *solid*; m.p. 190°C (dec.); $[\alpha]_D$ −3·0° (*c*, 0·96 in H_2O) (Found: C, 29·0; H, 5·4. $C_5H_{11}KO_6$ requires C, 29·1; H, 5·4%). δ_H (D_2O/MeOH) 4·06, 1H, ddd ≈ dt, $J_{2,3}$ 2.5, $J_{3,4}$ 6·5, $J_{3,4'}$ 6·5, H3; 3·93, 1H, d, H2; 3·77, 2H, m, H5, H5′; 1·84, 2H, m, H4, H4′. δ_H (D_2O/CH_3CN) 179·2, C1; 75·3, C2; 69·9, C3; 58·9, C5; 35·6, C4.

*Potassium Salt of 4-O-Methyl-*L*-arabinonic Acid Monohydrate (19)*

Compound (16) (760 mg, 4·63 mmol) was oxidized as described for (17). After crystallization at −20°C overnight and filtration, pure (19) (749 mg, 73%) was obtained as a white *solid*; m.p. 198°C (dec.); $[\alpha]_D$ +12·6° (*c*, 0·95 in H_2O) (Found: C, 31·0; H, 5·6. $C_6H_{13}KO_7$ requires C, 30·5; H, 5·6%). δ_H (D_2O/CH_3CN) 4·19, 1H, d, $J_{2,3}$ 2·0, H2; 4·00, 1H, dd, $J_{4,5}$ 2·5, $J_{5,5'}$ 12·5, H5; 3·96, 1H, dd, $J_{3,4}$ 9·0, H3; 3·74, 1H, dd, $J_{4,5'}$ 5·0, H5; 3·51, 3H, s, OMe; 3·43, 1H, ddd, H4. δ_C (D_2O/CH_3CN) 178·8, C1; 79·9, C2; 70·7, 69·7, C3, C4; 58·7, C5, 56·7, OMe.

*Potassium Salt of 4-Amino-4-deoxy-*D*-xylonic Acid Monohydrate (20)*

Compound (17) (58 mg, 0·23 mmol) was dissolved in distilled water (5 ml), and palladium on carbon (10%, 50 mg) was added. The mixture was stirred for 3 h in a hydrogen atmosphere. After filtration and freeze drying, (20) (44 mg, 85%) was obtained as a white *solid*; m.p. 168°C (dec.); $[\alpha]_D$ +12·8° (*c*, 0·76 in H_2O) (Found: C, 27·0; H, 5·5; N, 6·0, $C_5H_{12}KNO_6$ requires C, 27·1; H, 5·5; N, 6·3%). δ_H (D_2O/CH_3CN) 4·01, 1H, d, $J_{2,3}$ 2·5, H2; 3·87, 1H, dd, $J_{3,4}$ 6·5, H3; 3·70, 1H, dd, $J_{4,5}$ 4·5, $J_{5,5'}$ 11·5, H5; 3·58, 1H, dd, $J_{4,5'}$ 6·5, H5′; 3·13, 1H, ddd ≈ dt, H4. δ_C (D_2O/CH_3CN) 177·0, C1; 72·1, 71·1, C2, C3; 60·3, C5; 53·7, C4.

Potassium Salt of L*-Arabinonic Acid (21)*

This was prepared according to ref. 10.

*1,2,3,6-Tetra-*O*-benzoyl-4-deoxy-α-*D*-glucopyranose (25)*

Compound (25) was synthesized from D-galactose (22) according to ref. 8 via 1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose (23) and 1,2,3,6-tetra-*O*-benzoyl-4-deoxy-4-iodo-α-D-glucopyranose (24) in 11% overall yield.

*4-Deoxy-*D*-xylo-hexopyranose (26)*

Compound (25) (1·27 g, 2·19 mmol) was debenzoylated as described for (13). The reaction was stopped by the addition of Amberlite IR 120 H^+ cation-exchange resin. The resin was filtered off and the solvent was evaporated under vacuum. The crude residue was dissolved in water (20 ml), and extracted several times with toluene. The aqueous layer was removed by freeze drying and the crude product was purified by chromatography on Sephadex G 10 to give pure (26) (268 mg, 75%) as a white solid in an α:β ratio of 1:2·2. α-Anomer: δ_H (D_2O/CH_3CN) 5·35, 1H, d, $J_{1,2}$ 3·5, H1; 4·20, 1H, dddd, $J_{4ax,5}$ 12·0, $J_{4eq,5}$ 12·0, $J_{5,6}$ 3·0, $J_{5,6'}$ 6·0, H5; 4·05, 1H, ddd, $J_{2,3}$ 10·0, $J_{3,4ax}$ 11·0, $J_{3,4eq}$ 5·0, H3; 3·80–3·65, 2H, m, H6, H6′; 2·09, 1H, ddd, $J_{4ax,4eq}$ 12·5, H4*eq*; 1·54, 1H, ddd, H4*ax*. δ_C (D_2O/CH_3CN) 91·9, C1; 72·4, C2; 67·6, 66·0, C3, C5; 62·9, C6; 33·4, C4. β-Anomer: δ_H (D_2O/CH_3CN) 4·65, 1H, d, $J_{1,2}$ 8·0, H1; 3·84, 1H, ddd, $J_{2,3}$ 9·0, $J_{3,4ax}$ 11·0, $J_{3,4eq}$ 5·0, H3; 3·80–3·65, 3H, m, H5, H6, H6′; 3·25, 1H., dd, H2; 2·06, 1H, ddd, $J_{4eq,4ax}$ 13, $J_{4eq,5}$ 5·2, H4*eq*; 1·52, 1H, ddd, $J_{4ax,5}$ 12·0, H4*ax*. δ_C (D_2O/CH_3CN) 95·3, C1; 75·2, C2; 71·6, 69·5, C3, C5; 62·8, C6; 33·4, C4.

*Potassium Salt of 4-Deoxy-*D*-xylo-hexonic Acid Monohydrate (27)*

Compound (26) (236 mg, 1·44 mmol) was oxidized as described for (17). After crystallization for 24 h at 4°C and several washings with methanol, pure (27) (250 mg, 73·5%) was obtained as a white *solid*; m.p. 178°C (dec.); $[\alpha]_D$ −11.2° (*c*, 1·0 in H_2O) (Found: C, 30·3; H, 5·5. $C_6H_{13}KO_7$ requires C, 30·5; H, 5·5%). δ_H (D_2O/CH_3CN) 4·20, 1H, ddd ≈ t, $J_{2,3}$ 3·0, $J_{3,4}$ 10·0, $J_{3,4'}$ 3·0, H3; 3·95, 2H, m, H2, H5; 3·72, 1H, dd, $J_{5,6}$ 4·0, $J_{6,6'}$ 11·5, H6; 3·60, 1H, dd, $J_{5,6'}$ 7·0, H6′; 1·80, 1H, ddd, $J_{4,4'}$ 14·0, $J_{4,5}$ 3·0, H4; 1·67, 1H, ddd, $J_{4',5}$ 10·0, H4′. δ_C (D_2O/CH_3CN) 177·9, C1; 74·8, C2; 68·1, 67·7, C3, C5; 65·2, C6; 35·6, C4.

*Lithium Salt of 3-Deoxy-2-keto-*D*-threo-hexonic Acid Monohydrate (28)*

This was synthesized as previously described.[15]

Lithium Salt of 3,4-Dideoxy-2-ketopentonic Acid Monohydrate (29)

A solution of (18) (20·6 mg, 0·1 mmol) in TRIS buffer (pH 8, 20 ml) was treated at the beginning and after 3 and 6 days with three portions each of 1·5 U DHAD in TRIS buffer (pH 8, 12 ml), and was incubated overall for 9 days at 35°C. Proteins were removed by membrane filtration (cellulose acetate 0·45 μm, Satorius), and the solution was subjected to a column of Lewatit H^+. The fractions containing compound (29) were pooled and brought to pH 7·5 by addition of a 0·25 M of aqueous lithium hydroxide. After freeze drying, the crude product was passed through Sephadex G 10 to give pure (29) (11·4 mg, 83%) as a mixture of anomers (an unequivocal assignment of n.m.r. signals for the α and β configuration was not possible). Anomer 1: δ_H (D_2O/CH_3CN) 4·10, 1H, ddd ≈ dt, $J_{4(4'),5}$ 5·5 (7·5), $J_{5,5'}$ 7·5, H5; 4·02, 1H, ddd ≈ q, $J_{4(4'),5}$ 7·5 (7·5), H5′; 2·14–2·00, 4H, m, H3, H3′, H4, H4′. δ_C (D_2O/CH_3CN) 177·2, C1; 103·3, C2; 68·1, C5; 24·4, 23·2, C3, C4. Anomer 2: δ_H (D_2O/CH_3CN) 3·65, 2H, t, $J_{4(4'),5(5')}$ 6·5, H5, H5′; 2·83, 1H, ddd, $J_{3,3'}$ 11·0, $J_{3,4(4')}$ 3·0 (8·0), H3; 1·85, 3H, m, H3′, H4, H4′. δ_C (D_2O/CH_3CN) 169·8, C1; 113·2, C2; 68·1, C5; 23·2, 23·1, C3, C4.

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References

[1] Flint, D. H., and Emptage, M. H., in 'Biosynthesis of Branched Chain Amino Acids' (Eds Z. Barak, D. M. Chipman and S. V. Schloss) pp. 282–315 (VCH: Weinheim 1990).

[2] Wixom, R. L., Blankenship, J. W., and Kanamori, M., *Biochim. Biophys. Acta*, 1961, **53**, 433.

[3] Arfin, S. M., and Umbarger, H. E., *J. Biol. Chem.*, 1969, **244**, 1118; Arfin, S. M., *J. Biol. Chem.*, 1969, **244**, 2250.

[4] Flint, D. H., and Emptage, M. H., *J. Biol. Chem.*, 1988, **263**, 3558.

[5] Pirrung, M. C., Holmes, C. P., Horowitz, D. M., and Nunn, D. S., *J. Am. Chem. Soc.*, 1991, **113**, 1020.

[6] Armstrong, F. B., Hedgecock, C. J. R., Reary, J. B., Whithouse, D., and Crout, D. H. G., *J. Chem. Soc., Chem. Commun.*, 1974, 351. Armstrong, F. B., Müller, U. S., Reary, J. B., Whithouse, D., and Crout, D. H. G., *Biochim. Biophys. Acta*, 1977, **498**, 282; Armstrong, F. B., Lipscomb, E. L., Crout, D. H. G., and Morgan, P. J., *J. Chem. Soc., Perkin Trans. 1*, 1985, 691.

[7] Limberg, G., Klaffke, W., and Thiem, J., *Bioorg. Med. Chem.*, 1995, **3**, 487.
[8] Leon, B., Liemann, S., and Klaffke, W., *J. Carbohydr. Chem.*, 1993, **12**, 597.
[9] Sivakumaran, T., and Jones, J. K. N., *Can. J. Chem.*, 1967, **45**, 2493.
[10] Moore, S., and Link, K. P., *J. Biol. Chem.*, 1940, **133**, 293.
[11] Cramer, F., and Hennrich, H., *Chem. Ber.*, 1961, **94**, 976.
[12] Wessel, H. P., Iversen, T., and Bundle, D. R., *J. Chem. Soc., Perkin Trans. 1*, 1985, 2247.
[13] Baley, J. F., Bullock, C., O'Brien, E., and Williams, J. M., *Carbohydr. Res.*, 1975, **43**, 43.
[14] Lindhorst, T. K., Ph.D. Thesis, Hamburg, 1991.
[15] Limberg, G., and Thiem, J., *Carbohydr. Res.*, 1995, **275**, 107.
[16] MacGee, J., and Doudoroff, M., *J. Biol. Chem.*, 1954, **210**, 617.