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α-Azidoesters as Divergent Intermediates for Combinatorial Generation of Glucofuranose Libraries of Novel N-Linked Glycopeptides

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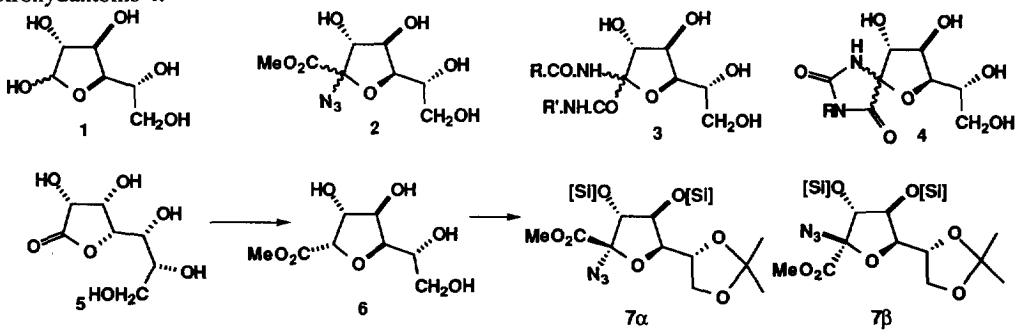
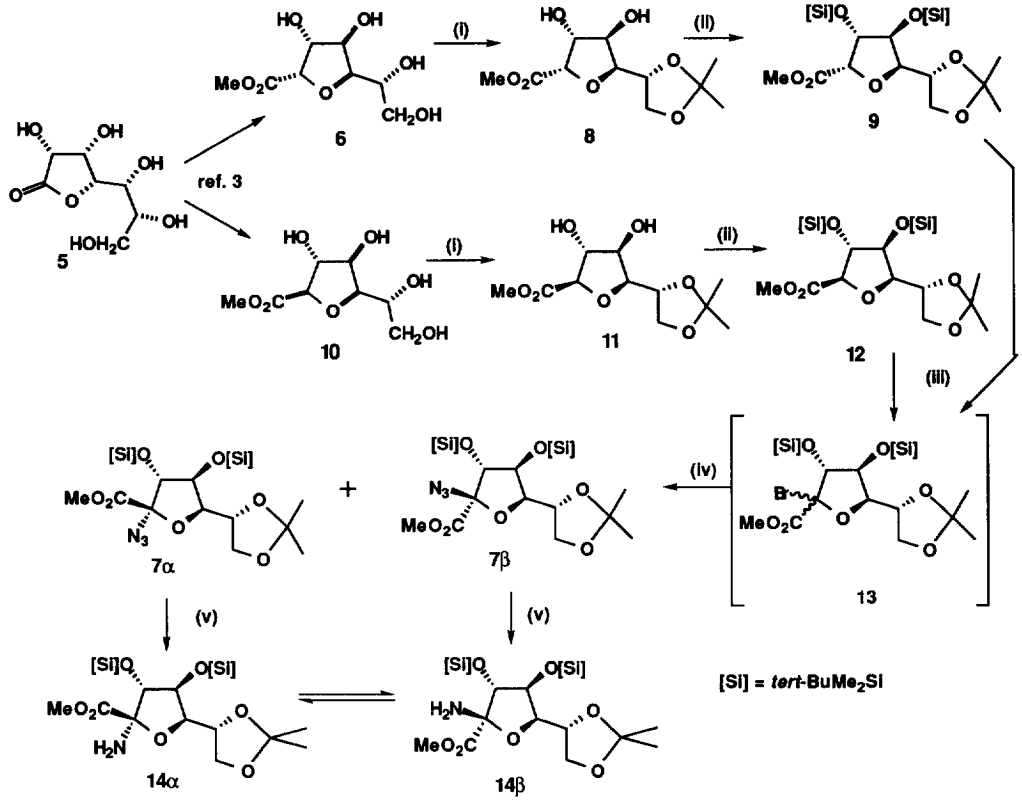
Abstract: Epimeric azidoesters containing a glucofuranosyl moiety are readily prepared from glucoheptonolactone and may be reduced to corresponding aminoesters; such compounds may be useful intermediates for the generation of combinatorial libraries of glucofuranose mimics and of spiroderivatives of glucofuranose at the anomeric position. The X-ray crystal structure of methyl 2-amino-2-deoxy-3,4-di-*O*-*tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate is reported.

Glucose is involved in a large number of biochemical processes; specific binding to enzymes or receptors that recognise glucose requires both potency and specificity. Structures that contain a glucose recognition site [which provides the binding] together with an opportunity for the synthesis of diverse structures [which should provide the specificity] could produce leads for the control of individual steps of glucose metabolism. *C*-glycosides are mimics of sugars which are likely to be metabolically stable.[1] Structures that incorporate a nitrogen function as well as a carboxyl group at the anomeric position can provide a library of materials that would allow the sugar epitope to be kept, as well as enabling the incorporation of a wide range of structural moieties which could be introduced by amide bonds formation at the anomeric position. Although glucose most commonly occurs in the pyranose form, there are several mimics of glucofuranose **1** which inhibit various enzymes involved in the metabolism of glucose.[2] Easy access to protected derivatives of the epimeric azidoesters **2** would allow the synthesis of families of materials such as novel N-linked glycopeptides **3** and of other derivatives at the anomeric position of glucofuranose exemplified by the spirohydantoins **4**.



This paper reports the synthesis of the epimeric azidoesters **7** from the *C*-glucofuranoside **6**,[3] which is readily prepared from the cheap[4] glucoheptonolactone **5**. Some of this work has been published in preliminary form.[5]



Scheme (i) Me_2CO, CSA (ii) *tert*-$BuMe_2SiCl$, imidazole, DMF (iii) NBS, $(PhCO)_2O$, CCl_4 (iv) NaN_3, DMF (v) H_2, Pd, MeOH

For the synthesis of the azides **7**, the readily available diacetonide[6] of glucoheptonolactone **5** was converted into the *C*-glucofuranoside **6** in an overall yield of 66% as previously described. The strategy for introduction of the azide [Scheme] is to exploit the relative stability of the captodative radical formed by abstraction of a hydrogen from C-2 in **6**;[7] for such radical halogenation, it is necessary to protect most of the free hydroxyl groups in **6**. Initial acetonation of the side chain diol in **6** with acetone in the presence of camphor sulfonic acid afforded **8** [80% yield]; subsequent reaction with *tert*-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole gave the fully protected ester **9** [80% yield]. Radical bromination[8] of **9** by N-bromosuccinimide in carbon tetrachloride in the presence of benzoyl peroxide as initiator gave two unstable epimeric bromides **13** which were treated with sodium azide in dimethylformamide to give a combined yield of 71% of the epimeric azides **7α** [37% yield] and **7β** [34% yield]. The overall yield of the azides **7** from the diacetonide of **5** is 30% and this provides convenient access to these compounds as potential intermediates for the synthesis of libraries of compounds that contain the glucofuranose. In some cases, epimeric carboxylate esters give very different results during radical bromination, so a similar series of reactions was carried out on the methyl ester **10**, epimeric at C-2 with **6**. Thus acetonation of **10** [69% yield], followed by silylation of the resulting diol **11** [71% yield] afforded the completely protected ester **12**, epimeric at C-2 with **9**. Sequential radical bromination and azide displacement of **12** gave the azides **7** in rather lower yield and slightly different relative proportions than resulted from **9**. Since **9** is far more easily available, the route from **9** to **7** is more experimentally convenient than that from **12**.

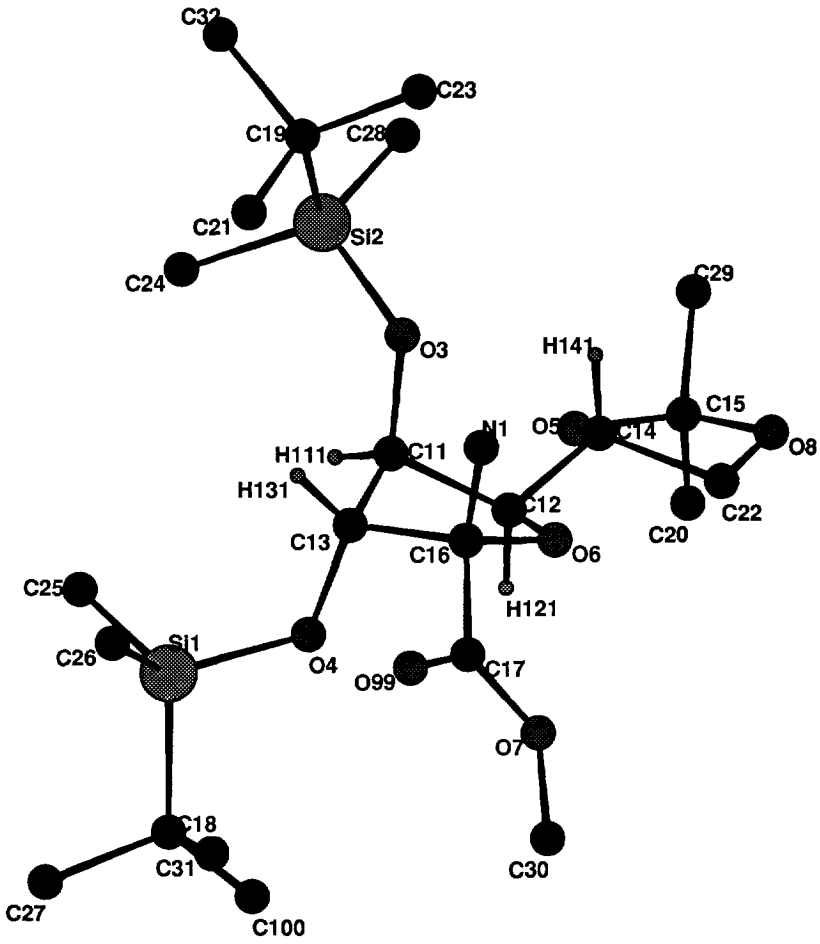


Figure X-Ray structure of methyl 2-amino-2-deoxy-3,4-di-*O*-*tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate **14β** showing crystallographic numbering scheme

The azides **7** are separable and do not interconvert. Hydrogenation of **7α** in methanol in the presence of palladium on carbon gave the epimeric amines **14**; **14α** [61% yield] may be separated from **14β** [28% yield] by column chromatography. Hydrogenation of **7β** under the same conditions gave 34% of **14α** and 60% of **14β**. The anomeric amines **14** interconvert slowly - presumably *via* an open chain imine - and so the relative proportions of the anomers may vary with the length of time and precise details of the column separation; at equilibrium, the ratio of the amines is approximately 1:1. Nonetheless, pure samples of the amines may be obtained, although there may be many circumstances where the use of a mixture of the amines would suffice. The structure of **14β** was firmly established by single crystal X-ray crystallographic analysis and is the first example of a crystal structure of a simple amino acid derivative in which the α-carbon of the aminoacid is also the anomeric of a sugar.

Sugar mimics with both an N-acyl group and a carbonyl function at the anomeric position possess a carbohydrate epitope and additionally allow substantial chemical diversity to be introduced at the anomeric position; even though amines such as **14** undergo easy epimerisation, N-acyl derivatives are configurationally stable. The epimeric azidoesters **7** and aminoesters **14** should allow the preparation of combinatorial libraries of compounds containing the glucofuranosyl moiety for high through-put screening of glucofuranose mimics. The generation of libraries of such materials by combinatorial technology and subsequent screening allow the discovery of glucofuranose mimics with highly specific biological activity. Similar approaches to the synthesis of combinatorial libraries containing L-rhamnose[9] and D-mannose[10] recognition sites have been reported. The azidoesters **7** are also suitable starting materials for the synthesis of other novel derivatives of glucofuranose and the following paper reports the synthesis of some spirohydantoins of glucofuranose as part of a project involved with the design of inhibitors of glycogen phosphorylase for the treatment of late onset diabetes.[11]

Acknowledgment: Support has been received for Graduate Studentships and Post-doctoral fellowships from EPSRC, Deutsche Forschungsgemeinschaf, Human Capital & Mobility Nr ERB4001GT933084, and EEC contract BIO2 CT94 3025.

Fractional atomic coordinates and equivalent isotropic temperature factors U(iso) with standard deviations in parentheses for the amino ester **14β**:

Atom	*x/a*	*y/b*	*z/c*	*U(equiv)*
Si(1)	0.51755(7)	-0.28424(5)	0.68326(3)	0.0530
Si(2)	0.31391(6)	-0.01938(5)	0.93424(2)	0.0480
O(3)	0.4301(2)	0.0194(1)	0.85585(7)	0.0518
O(4)	0.5416(2)	-0.1469(1)	0.68649(7)	0.0507
O(5)	0.1497(2)	0.1902(1)	0.7366(1)	0.0604
O(6)	0.6260(2)	0.0984(1)	0.71274(9)	0.0540
O(7)	0.8452(2)	0.0172(1)	0.60917(9)	0.0684
O(8)	0.2550(2)	0.3643(1)	0.7093(2)	0.0773
O(99)	0.9830(2)	-0.1032(2)	0.6879(1)	0.0760
N(1)	0.8371(2)	0.0271(2)	0.8071(1)	0.0641
C(11)	0.4088(2)	-0.0158(2)	0.77810(9)	0.0445
C(12)	0.4282(2)	0.0822(1)	0.72183(9)	0.0465
C(13)	0.5787(2)	-0.0867(1)	0.75485(9)	0.0452
C(14)	0.3493(2)	0.1917(1)	0.7485(1)	0.0498
C(15)	0.0901(3)	0.3021(2)	0.7207(1)	0.0591
C(16)	0.7288(2)	0.0038(1)	0.7383(1)	0.0484
C(17)	0.8656(2)	-0.0341(2)	0.6758(1)	0.0539
C(18)	0.5083(3)	-0.3184(2)	0.5767(1)	0.0703
C(19)	0.4978(3)	-0.0380(2)	1.0128(1)	0.0591
C(20)	-0.0323(4)	0.2995(3)	0.6499(2)	0.0882
C(21)	0.6485(4)	-0.1206(2)	0.9860(2)	0.0781
C(22)	0.4078(3)	0.2910(2)	0.7005(1)	0.0583
C(23)	0.5933(4)	0.0718(2)	1.0305(1)	0.0761
C(24)	0.1814(3)	-0.1492(2)	0.9157(1)	0.0674
C(25)	0.3006(4)	-0.3272(2)	0.7334(2)	0.0795
C(26)	0.7186(5)	-0.3517(3)	0.7323(2)	0.0924
C(27)	0.4983(5)	-0.4436(3)	0.5651(2)	0.0941
C(28)	0.1481(3)	0.0930(2)	0.9618(1)	0.0700
C(29)	-0.0076(5)	0.3490(3)	0.7904(2)	0.0907
C(30)	0.9797(4)	-0.0108(3)	0.5505(2)	0.0876
C(31)	0.3373(5)	-0.2646(3)	0.5391(2)	0.1001
C(32)	0.4083(5)	-0.0833(3)	1.0860(2)	0.0944
C(100)	0.6830(5)	-0.2717(4)	0.5364(2)	0.1010

X-Ray Crystal Analysis The absolute configuration of the chiral centres in methyl 2-amino-2-deoxy-3,4-di-*O*-*tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate **14β** (crystallised from ethyl acetate:hexane, 1:3) was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer. A suitable crystal of approximate dimensions 0.20 x 0.45 x 0.50 mm was selected. Molecular formula $C_{23}H_{47}Si_2O_7N$. Formula weight 505.80; Monoclinic P 21. No. of molecular units in the cell Z, 2. Calculated Density (gcm^{-3}), 1.14. Reflections for lattice parameters, 25. θ range for lattice parameters, 19.00 - 43.00. Linear absorption coeff.(cm-1), 1.39. Data collection parameters: h range, -7 to 7; k range, -11 to 11;
range, 0 to 17; θ range, 0 to 74.33° Copper radiation, λ = 1.5418Å. Temperature (K), 294. No. of intensity standards, 3. Decay of standards, 8.27%. Total data collected, 5055. Number of reflections used, 5041. Criterion for observed, $I > 3\sigma(I)$. The data were corrected for absorption, Lorentz and polarisation effects. All calculations were carried out on a 486PC computer. SIR92 succeeded in finding all 33 non-hydrogen atoms in the molecule and these were put into CRYSTALS[12] as 23 carbon, 2 silicon, 1 nitrogen, and 7 oxygen atoms; these were refined to convergence, using full matrix least-squares refinement, of the positional parameters and isotropic temperature factors. Atomic scattering factors were taken from International Tables.[13] The hydrogen atoms were placed geometrically and refinement completed with all the non-hydrogen atom temperature factors refined anisotropically. Corrections for secondary extinction and anomalous scattering were applied.[14] Refinement: Maximum number of parameters, 440. Ratio of data : parameters, 11.45:1. Flack enantiopole parameter, 0.03(3). The data were refined using Chebyshev three term weighting scheme[15] to give a final value of R = 0.048, Rw = 0.045. Cell parameters 7.151(2), 12.028(4), 17.227(6) Å. Atomic coordinates for the compound have been deposited at the Cambridge Crystallographic Data Centre.[16]

Experimental: Melting points were recorded on a Kofler hot block and are corrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz) or a Bruker AM 500 (500 MHz) spectrometer. ^{13}C Nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz), a Bruker AC 200 (50 MHz) or a Bruker AM 500 (125 MHz) spectrometer and multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; app, apparent. Infra-red spectra were recorded on a Perkin-Elmer 1750 IR FT spectrophotometer. Mass spectra were recorded on a VG Masslab 20-250, BIO-Q or using desorption chemical ionisation (DCI NH_3), chemical ionisation (CI NH_3), electrospray or thermospray, as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Microanalyses were performed by the microanalysis service of the Dyson Perrins laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica, and plates were developed using a spray of 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate in 2M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; hexane was distilled at 68°C before use to remove less volatile fractions. **D**-*glycero*-**D**-*gulo*-Heptono-1,4-lactone **5** was purchased from Sigma and converted into the *C*-glucofuranosides **6** and **10** as previously described.[3]

Methyl 2,5-anhydro-6,7-O-isopropylidene-D-glycero-D-ido-heptonate 8: Methyl 2,5-anhydro-D-*glycero*-D-*ido*-heptonate **6** (2.64 g, 11.9 mmol) was suspended in dry acetone (50 ml). After addition of camphor sulfonic acid (140 mg, 0.60 mmol) the mixture was heated to 50°C for 2.5 h when t.l.c. (ethyl acetate/methanol 4:1) showed only a trace of starting material (R_f 0.4) and the formation of one product (R_f 0.7). After cooling to room temperature sodium bicarbonate (100 mg, 1.20 mmol) was added, the mixture was stirred for 10 min, filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (ethyl acetate) to afford *the title compound 8* (2.49 g, 80%) as a white solid, m.p. 103-104°C. (Found: C, 50.12; H, 6.72%. $C_{11}H_{18}O_7$ requires C, 50.38; H, 6.92%). $[\alpha]_D^{25}$ -34.9 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 3463, 3402 cm^{-1} (OH), 1747 cm^{-1} (C=O). m/z (CI NH_3): 280 ($M+NH_4^+$, 80%), 263 (MH^+, 100%). δ_H (500 MHz, $CDCl_3$): 1.37, 1.44 (2 s, 2 x 3 H, CMe_2), 2.60 (d, J = 4.9 Hz, 1 H, OH), 2.83 (d, J = 2.9 Hz, 1 H, OH), 3.81 (s, 3 H, OMe), 4.04 (dd, J = 5.3, 8.7 Hz, 1 H, H-7), 4.11 (dd, J = 6.2, 8.7 Hz, 1 H, H-7'), 4.24 (dd, J = 3.3, 7.9 Hz, 1 H, H-5), 4.33-4.37 (m, 2 H, H-4, H-6), 4.49 (t, J = 3.9 Hz, 1 H, H-3), 4.81 (d, J = 3.9 Hz, 1 H, H-2). δ_C (50 MHz, $CDCl_3$): 25.1, 26.5 (2 q, $C\underline{Me}_2$), 52.1 (q, OMe), 67.1 (t, C-7), 73.2, 76.4, 78.3, 80.7, 82.1 (5 d, C-2, C-3, C-4, C-5, C-6), 109.1 (s, $\underline{C}Me_2$), 171.0 (s, C=O).

Methyl 2,5-anhydro-2,3-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-D-glycero-D-ido-heptonate 9: Methyl 2,5-anhydro-6,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptonate **8** (1.07 g, 4.09 mmol) was dissolved in dry dimethylformamide (15 ml). Imidazole (1.12 g, 16.4 mmol) and *tert*-butyldimethylsilyl chloride (1.85 g, 12.3 mmol) were added and the mixture was heated to 65°C for 24 h when t.l.c. (ethyl acetat/hexane 1:2) showed no starting material (R_f 0.4) and the formation of one product (R_f 0.8). After cooling to room temperature, the solvent was removed under reduced pressure and the the residue was coevaporated with toluene and purified by flash chromatography (diethyl ether/hexane 1:9) to afford *the title compound 9* (1.60 g, 80%) as a white solid, m.p. 65-66°C. (Found: C, 56.56; H, 9.22%. $C_{23}H_{46}O_7Si_2$ requires C, 56.29; H, 9.45%). $[\alpha]_D^{25}$ -32.0 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 1732 cm^{-1} (C=O). m/z (DCI NH_3): 508 ($M+NH_4^+$, 100%), 491 (MH^+, 35%). δ_H (500 MHz, $CDCl_3$): 0.06, 0.12 (2 s, 2 x 3 H, SiMe), 0.14 (s, 6 H, SiMe), 0.86, 0.91 (2 s, 2 x 9 H, $SiCMe_3$), 1.33, 1.40 (2 s, 2 x 3 H, CMe_2), 3.73 (s, 3 H, OMe), 4.07 (m, 1 H, H-4), 4.09 (dd, J = 5.5, 8.3 Hz, 1 H, H-7), 4.13 (dd, J = 5.9, 8.3 Hz, 1 H, H-7'), 4.15 (dd, J = 2.5, 8.6 Hz, 1 H, H-5), 4.20 (app dt, J = 5.7, 8.6 Hz, 1 H, H-6), 4.27 (dd, J = 1.1, 3.4 Hz, 1 H, H-3), 4.70 (d, J = 3.4 Hz, 1 H, H-2). δ_C (50 MHz, $CDCl_3$): -5.3, -5.2, -4.8, -4.6 (4 q, SiMe) 17.7, 18.0 (2 s, $Si\underline{C}Me_3$), 25.4, 25.6, 26.8 (3 q, $SiC\underline{Me}_3$, $C\underline{Me}_2$), 51.6 (q, OMe), 67.7 (t, C-7), 72.4, 77.1, 79.4, 81.6, 83.3 (5 d, C-2, C-3, C-4, C-5, C-6), 108.8 (s, $\underline{C}Me_2$), 171.0 (s, C=O).

Methyl 2-azido-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-α-D-gluco-2-heptulofuranosonate 7α and methyl 2-azido-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-β-D-gluco-2-heptulofuranosonate 7β: Method I (from 9): Methyl 2,5-anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptonate **9** (1.59 g, 3.25 mmol) was dissolved in dry carbon tetrachloride (40 ml) under an atmosphere of nitrogen . Benzoyl peroxide (39 mg, 0.16 mmol) and *N*-bromosuccinimide (751 mg, 4.23 mmol) were added and the mixture was heated to 80°C for 30 min, when t.l.c. (ethyl acetate/hexane 1:4) showed only a trace of starting material (R_f 0.6) and the formation of two

major products (R_f 0.62, 0.63). The mixture was cooled to room temperature, filtered and the solvent was removed under reduced pressure. The residue was dissolved in dry dimethylformamide (10 ml) and sodium azide (250 mg, 3.85 mmol) was added. After stirring for 20 h the solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and water. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (diethyl ether/hexane 1:10) to afford *methyl 2-azido-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-α-D-gluco-2-heptulofuranosonate 7α* (643 mg, 37%) as a colourless oil. (Found: C, 51.60; H, 8.63; N, 7.90%. $C_{23}H_{45}N_3O_7Si_2$ requires C, 51.95; H, 8.53; N, 7.90%). $[\alpha]_D^{25}$ -28.4 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 2127 cm^{-1} (N_3), 1773, 1749 cm^{-1} (C=O). m/z (CI NH_3): 549 ($M+NH_4^+$, 56%), 504 (MH^+, 84%), 90 (100%). δ_H (500 MHz, $CDCl_3$): 0.08, 0.11, 0.18, 0.22 (4 s, 4 x 3 H, SiMe), 0.87, 0.96 (2 s, 2 x 9 H, $SiCMe_3$), 1.33, 1.42 (2 s, 2 x 3 H, CMe_2), 3.75 (s, 3 H, OMe), 4.09 (dd, J = 1.1, 2.5 Hz, 1 H, H-4), 4.11 (dd, J = 4.5, 8.6 Hz, 1 H, H-7), 4.20 (dd, J = 5.5, 8.6 Hz, 1 H, H-7'), 4.28 (app dt, J = 5.0, 8.5 Hz, 1 H, H-6), 4.30 (dd, J = 2.5, 8.5 Hz, 1 H, H-5), 4.46 (d, J = 1.1 Hz, 1 H, H-3). δ_C (50 MHz, $CDCl_3$): -5.6, -5.3, -5.1 (3 q, SiMe) 17.9, 18.0 (2 s, $SiCMe_3$), 25.1, 25.4, 25.5, 26.8 (4 q, $SiCMe_3$, CMe_2), 52.8 (q, OMe), 67.6 (t, C-7), 72.1, 76.9, 82.2, 85.2 (4 d, C-3, C-4, C-5, C-6), 97.3 (s, C-2), 109.2 (s, CMe_2), 167.9 (s, C=O).

Further elution of the column gave *methyl 2-azido-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-β-D-gluco-2-heptulofuranosonate 7β* (591 mg, 34%) as a colourless oil. (Found: C, 51.99; H, 8.54; N, 7.42%. $C_{23}H_{45}N_3O_7Si_2$ requires C, 51.95; H, 8.53; N, 7.90%). $[\alpha]_D^{25}$ -80.8 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 2130 cm^{-1} (N_3), 1769, 1753 cm^{-1} (C=O). m/z (CI NH_3): 549 ($M+NH_4^+$, 52%), 504 (MH^+, 72%), 90 (100%). δ_H (500 MHz, $CDCl_3$): 0.07, 0.13, 0.16, 0.17 (4 s, 4 x 3 H, SiMe), 0.86, 0.95 (2 s, 2 x 9 H, $SiCMe_3$), 1.35, 1.41 (2 s, 2 x 3 H, CMe_2), 3.78 (s, 3 H, OMe), 4.12 (d, J = 2.9 Hz, 1 H, H-4), 4.14 (dd, J = 5.0, 8.7 Hz, 1 H, H-7), 4.19 (dd, J = 5.9, 8.7 Hz, 1 H, H-7'), 4.23 (s, 1 H, H-3), 4.31 (dd, J = 2.9, 9.2 Hz, 1 H, H-5), 4.37 (app dt, J = 5.3, 9.2 Hz, 1 H, H-6), δ_C (50 MHz, $CDCl_3$): -5.8, -4.7, (2 q, SiMe), 17.6, 18.1 (2 s, $SiCMe_3$), 25.2, 25.5, 26.8 (3 q, $SiCMe_3$, CMe_2), 52.5 (q, OMe), 67.5 (t, C-7), 71.7, 76.8, 83.4, 86.5 (4 d, C-3, C-4, C-5, C-6), 97.9 (s, C-2), 109.1 (s, CMe_2), 166.6 (s, C=O).

Method II (from 12): Methyl 2,5-anhydro-2,3-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonate **12** (98 mg, 0.20 mmol) was dissolved in dry carbon tetrachloride (5 ml) under an atmosphere of nitrogen. Benzoyl peroxide (2.5 mg, 0.01 mmol) and *N*-bromosuccinimide (46 mg, 0.26 mmol) were added and the mixture was heated to 80°C for 80 min when t.l.c. (ethyl acetate/hexane 1:4) showed only a trace of starting material (R_f 0.6) and the formation of a major product (R_f 0.7). The mixture was cooled to room temperature, filtered and the solvent was removed under reduced pressure. The residue was dissolved in dry dimethyl formamide (3 ml) and sodium azide (20 mg, 0.3 mmol) was added. After stirring for 20 h the solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and water. The organic layer was separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (diethyl ether/hexane 1:10) to afford methyl 2-azido-2-deoxy-3,4-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-α-D-*gluco*-2-heptulofuranosonate **7α** (32 mg, 30%) identical to the material described above. Further elution of the coloun gave methyl 2-azido-2-deoxy-3,4-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate **7β** (17 mg, 16%) identical to the material described above.

Methyl 2,5-anhydro-6,7-O-isopropylidene-D-glycero-D-gulo-heptonate ***11****:* Methyl 2,5-anhydro-D-*glycero*-D-*gulo*-heptonate **10** (770 mg, 3.45 mmol) was suspended in dry acetone (25 ml). Camphor sulphonic acid (50 mg) was added and the mixture was stirred for 40 min when t.l.c. (ethyl acetate/methanol 10:1) showed no starting material (R_f 0.2) and one major product (R_f 0.8). Sodium bicarbonate (110 mg) was added and stirring was continued for 10 min. The mixture was filtered, the solvent was removed under reduced pressure and the residue was purified by flash chromatography (ethyl acetate/hexane 3:1) to give *the title compound* ***11*** (624 mg, 69%) as a colourless solid, m.p. 87-89°C. (Found: C, 50.38; H, 6.96%. $C_{11}H_{18}O_7$ requires C, 50.38; H, 6.92%). $[\alpha]_D^{25}$ -41.6 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 3465 cm^{-1} (br, OH), 1752, 1735 cm^{-1} (C=O), m/z (CI NH_3): 280 ($M+NH_4^+$, 72%), 263 (MH^+, 100%). δ_H (500 MHz, $CDCl_3$): 1.38, 1.45 (2 s, 2 x 3 H, CMe_2), 2.52, 2.93 (2 s, br, 2 x 1 H, OH), 3.79 (s, 3 H, OMe), 4.07 (dd, J = 4.9, 8.7 Hz, 1 H, H-7), 4.14 (dd, J = 3.5, 8.2 Hz, 1 H, H-5), 4.20-4.21 (m, 1 H, H-4), 4.21 (dd, J = 6.1, 8.7 Hz, 1 H, H-7'), 4.40 (d, J = 1.6 Hz, 1 H, H-2), 4.46 (ddd, J = 4.9, 6.1, 8.2 Hz, 1 H, H-6), 4.49 (app t, J = 1.5 Hz, 1 H, H-3). δ_C (50 MHz, $CDCl_3$): 25.1, 26.6 (2 q, $C\underline{Me}_2$), 52.5 (q, OMe), 67.3 (t, C-7), 73.0, 76.4, 80.7, 83.1, 83.5 (5 d, C-2, C-3, C-4, C-5, C-6), 109.3 (s, $\underline{C}Me_2$), 172.2 (s, C=O).

Methyl 2,5-anhydro-3,4-di-O-tert-butyldimetylsilyl-6,7-O-isopropylidene-D-glycero-D-gulo-heptonate ***12****:* Methyl 2,5-anhydro-6,7-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptonate **11** (296 mg, 1.13 mmol) was dissolved in dry dimethylformamide (10 ml). Imidazole (380 mg, 5.60 mmol) and *tert*-butyldimethylsilyl chloride (678 mg, 4.50 mmol) were added and the mixture was heated to 65°C for 24 h when t.l.c. (ethyl acetate/hexane 1:4) showed no starting material (R_f 0.0) and the formation of one product (R_f 0.6). After cooling to room temperature the solvent was removed under reduced pressure and the the residue was co-evaporated with toluene. The residue was dissolved in ethyl acetate (25 ml), washed with pH 7 buffer solution (10 ml), dried ($MgSO_4$) and purified by flash chromatography (diethyl ether/hexane 1:9) to afford *the title compound* ***12*** (394 mg, 71%) as a colouless oil. (Found: C, 56.05; H, 9.62%. $C_{23}H_{46}O_7Si_2$ requires C, 56.29; H, 9.45%). $[\alpha]_D^{25}$ -20.8 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 1770, 1734 cm^{-1} (C=O). m/z (CI NH_3): 508 ($M+NH_4^+$, 24%), 491 (MH^+, 21%). δ_H (500 MHz, $CDCl_3$): 0.08, 0.10, 0.14, 0.17 (4 s, 4 x 3 H, SiMe), 0.86, 0.91 (2 s, 2 x 9 H, $SiCMe_3$), 1.34, 1.42 (2 s, 2 x 3 H, CMe_2), 3.71 (s, 3 H, OMe), 3.97 (dd, J = 1.1, 2.9 Hz, 1 H, H-4), 4.08-4.11, (m, 2 H, H-5, H-7), 4.18 (dd, J = 6.2, 8.4 Hz, 1 H, H-7'), 4.30 (s, J = 5.7, 1 H, H-2), 4.32 (app dt, J = 6.2, 8.4 Hz, 1 H, H-6), 4.41 (br s, 1 H, H-3). δ_C (50 MHz, $CDCl_3$): -5.3, -5.0, -4.8 (3 q, SiMe) 17.8 (s, $Si\underline{C}Me_3$), 23.3, 25.5, 25.6, 26.8 (4 q, $SiC\underline{Me}_3$, $C\underline{Me}_2$), 51.8 (q, OMe), 67.6 (t, C-7), 72.6, 76.7, 82.1, 84.0, 84.4 (5 d, C-2, C-3, C-4, C-5, C-6), 108.6 (s, $\underline{C}Me_2$), 170.7 (s, C=O).

Methyl 2-amino-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-α-D-gluco-2-heptulofuranosonate ***14α*** *and methyl 2-amino-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-β-D-gluco-2-heptulofuranosonate* ***14β****:* A solution of methyl 2-azido-2-deoxy-3,4-di-*O*-*tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-α-D-*gluco*-2-heptulofuranosonate **7α** (680 mg, 1.23 mmol) in dry methanol (25 ml) was added to palladium on carbon (230 mg) in methanol (35 ml) under an atmosphere of hydrogen and the mixture was stirred for 3.5 h when t.l.c. (ethyl acetate/hexane 1:4) showed no starting material (R_f 0.7) and the formation of two major products (R_f 0.13, 0.22). The mixture was filtered through *Celite* and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/hexane 1:4) to afford *methyl 2-amino-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-*

6,7-O-isopropylidene-α-D-gluco-2-heptulofuranosonate ***14α*** (392 mg, 61%) as a colourless oil. (Found: C, 54.34; H, 9.46; N, 2.64%. $C_{23}H_{47}NO_7Si_2$ requires C, 54.62; H, 9.34; N, 2.77%). $[\alpha]_D^{25}$ -15.8 (*c*, 1.0 in $CHCl_3$). ν_{max} (film) 3405, 3333 cm^{-1} (NH_2), 1752 cm^{-1} (C=O). m/z (CI NH_3): 506 (MH^+, 66%), 474 (MH^+-HOMe, 41%), 374 (100%). δ_H (500 MHz, $CDCl_3$): 0.11, 0.12, 0.18, 0.19 (4 s, 4 x 3 H, SiMe), 0.88, 0.94 (2 s, 2 x 9 H, $SiCMe_3$), 1.32, 1.40 (2 s, 2 x 3 H, CMe_2), 2.24 (s, 2 H, NH_2), 3.73 (s, 3 H, OMe), 4.03 (dd, J = 5.5, 8.4 Hz, 1 H, H-7), 4.05 (dd, J = 1.1, 3.0 Hz, 1 H, H-4), 4.11 (dd, J = 3.0, 8.3 Hz, 1 H, H-5), 4.14 (dd, J = 6.2, 8.4 Hz, 1 H, H-7'), 4.22 (app dt, J = 5.8, 8.3 Hz, 1 H, H-6), 4.43 (d, J = 1.1 Hz, 1 H, H-3). δ_C (50 MHz, $CDCl_3$): -5.3, -4.9, -4.7 (3 q, SiMe), 18.1 (s, $SiCMe_3$), 25.3, 25.7, 25.8 (3 q, $SiCMe_3$, CMe_2), 52.4 (q, OMe), 67.6 (t, C-7), 72.6, 77.4, 79.6, 82.2 (4 d, C-3, C-4, C-5, C-6), 94.5 (s, C-2), 108.7 (s, CMe_2), 171,0 (s, C=O).

Further elution of the column with ethyl acetate/hexane 1:3 gave *methyl 2-amino-2-deoxy-3,4-di-O-tert-butyldimethylsilyl-6,7-O-isopropylidene-β-D-gluco-2-heptulofuranosonate* ***14β*** (183 mg, 28%) as a white solid, m.p. 76-78°C. (Found: C, 54.52; H, 9.69; N, 2.80%. $C_{23}H_{47}NO_7Si_2$ requires C, 54.62; H, 9.37; N, 2.77%). $[\alpha]_D^{25}$ -43.3 (*c*, 1.0 in $CHCl_3$). ν_{max} (KBr) 3358, 3313 cm^{-1} (NH_2), 1744 cm^{-1} (C=O). m/z (CI NH_3): 506 (MH^+, 100%), 474 (MH^+-HOMe, 50%), 374 (95%). δ_H (500 MHz, $CDCl_3$): 0.08 (2 s, 2 x 3 H, SiMe), 0.17 (s, 6 H, SiMe), 0.86, 0.93 (2 s, 2 x 9 H, $SiCMe_3$), 1.33, 1.39 (2 s, 2 x 3 H, CMe_2), 2.41 (s, 2 H, NH_2), 3.75 (s, 3 H, OMe), 4.04 (dd, J = 0.7, 2.7 Hz, 1 H, H-4), 4.10 (dd, J = 5.2, 8.6 Hz, 1 H, H-7), 4.14-4.18 (m, 3 H, H-3, H-5, H-7'), 4.27 (app dt, J = 5.6, 9.0 Hz, 1 H, H-6). δ_C (50 MHz, $CDCl_3$): -5.3, -5.1, -4.9, -4.6 (4 q, SiMe), 17.6, 18.1 (2 s, $SiCMe_3$), 25.5, 25.7, 26.8 (3 q, $SiCMe_3$, CMe_2), 52.4 (q, OMe), 67.8 (t, C-7), 72.4, 77.7, 83.5, 84.4 (4 d, C-3, C-4, C-5, C-6), 98.1 (s, C-2), 108.9 (s, CMe_2), 169.9 (s, C=O).

Under the same conditions methyl 2-azido-2-deoxy-3,4-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate **7β** (284 mg, 0.53 mmol) gave methyl 2-amino-2-deoxy-3,4-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-α-D-*gluco*-2-heptulofuranosonate **14α** (92 mg, 34%) and methyl 2-amino-2-deoxy-3,4-di-*O-tert*-butyldimethylsilyl-6,7-*O*-isopropylidene-β-D-*gluco*-2-heptulofuranosonate **14β** (162 mg, 60%).

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