Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 22, pp. 3807–3820, 1997

Printed in Great Britain
PII: S0957-4166(97)00561-2
0957-4166/97 $17.00 + 0.00

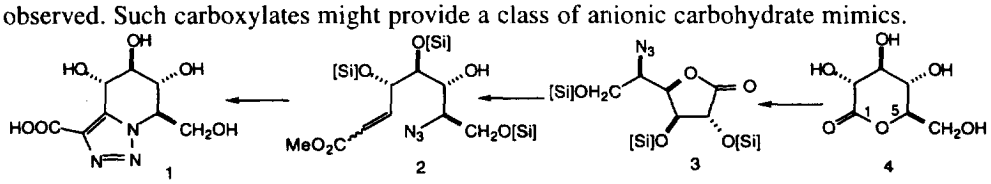
Triazole carboxylic acids as anionic sugar mimics? Inhibition of glycogen phosphorylase by a D-glucotriazole carboxylate

Thomas M. Krülle,[a] Carmen de la Fuente,[a] Lea Pickering,[a] Robin T. Aplin,[a] Katerina E. Tsitsanou,[b] Spyros E. Zographos,[b] Nikos G. Oikonomakos,[b] Robert J. Nash,[c] Rhodri C. Griffiths [c] and George W. J. Fleet [a,*]

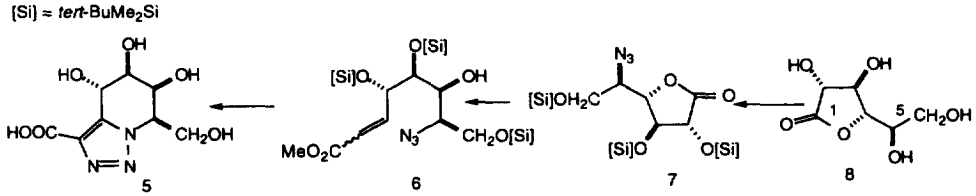
[a] Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK
[b] Institute of Biological Research and Biotechnology, The National Hellenic Research Foundation, 48 Vas. Constantinou Avenue, Athens 11635, Greece
[c] Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Cardiganshire SY23 3EB, UK

Abstract: Triazole-carboxylic acids related to D-glucose and D-galactose may be prepared by intramolecular [1,3]-dipolar cycloadditions of azides to unsaturated esters, followed by bromine oxidation of the resulting triazoline. Such materials may provide a series of anionic mimics of carbohydrates. © 1997 Elsevier Science Ltd

Tetrazoles,[1] triazoles[2] and imidazoles[3] which are fused to pyranoses and furanoses have been studied as inhibitors of various glycosidases. Tetrazoles, but not triazoles, are good inhibitors of some retaining β-glucosidases. Vasella has suggested that the cleavage of β-glycosides involves protonation at C1–O of the substrate in the plane of the pyranose ring.[4] Structure–activity relationships of such systems against β-glucosidases have been discussed.[5] We considered that such fused frameworks would also easily allow the preparation of carbohydrate analogues bearing a group off the aromatic heterocyclic ring which could form anions, allowing negatively charged sugar mimics to be prepared. This paper describes the synthesis of the triazole carboxylic acids related to D-glucose **1** and D-galactose **5**. Large scale and convenient routes to the azidolactones **3** and **7** involve initial introduction of an azide function with retention of configuration at C-5 of D-*glucono*-lactone **4** or of D-*galactono*-lactone **8**. Subsequent elaboration of C-1 by a Wittig extension provides efficient access to the azidoesters **2** and **6**, respectively, which undergo intramolecular [1,3]-dipolar cycloadditions to form the required bicyclic frameworks. The triazole acids **1** and **5** were shown to have no effect on a number of glycosidases with pH optima ranging from 4.0 to 7.3, indicating that neither the acid nor the carboxylate had any significant binding to the hydrolase. In contrast, weak but competitive inhibition of glycogen phosphorylase by the D-*gluco*-carboxylic acid **1** at pH 6.8, where **1** will exist as the carboxylate anion, was observed. Such carboxylates might provide a class of anionic carbohydrate mimics.



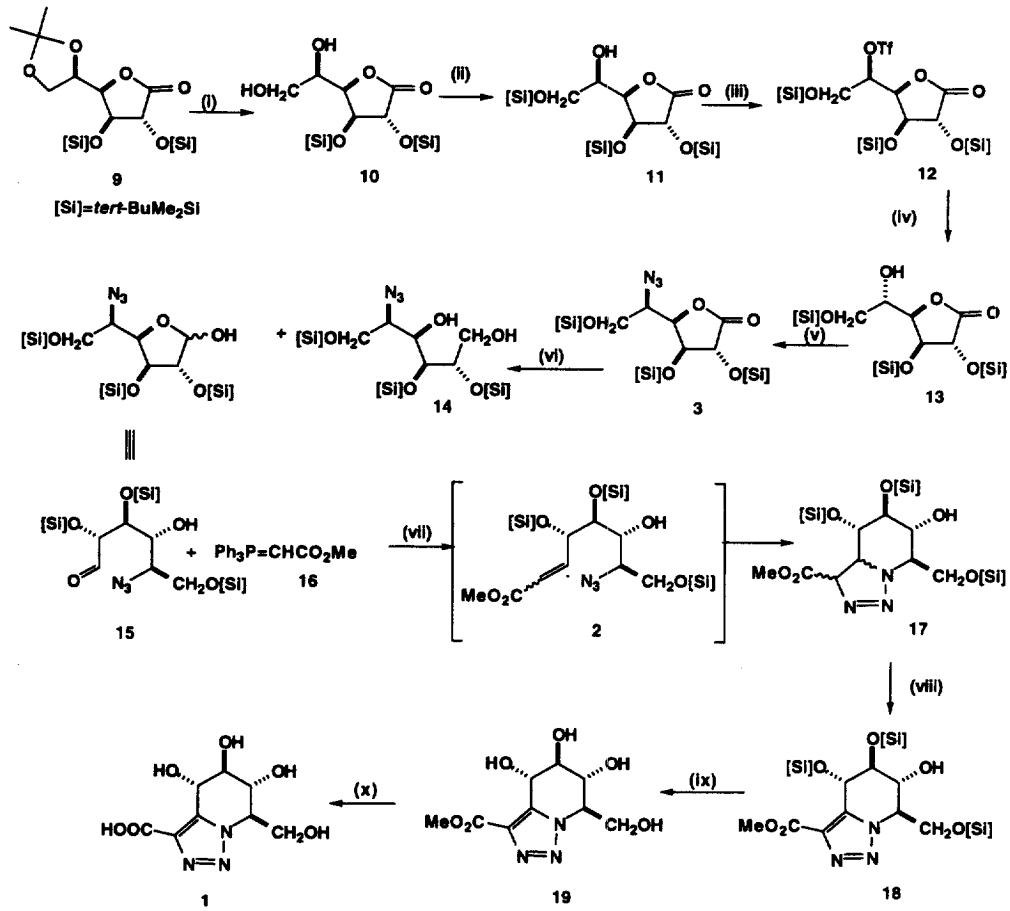
[Si] = *tert*-$BuMe_2Si$



For the synthesis of the D-*gluco*-triazole carboxylic acid **1**, it is necessary to introduce an azide functionality at C-5 of D-*glucono*-lactone **4** with retention of configuration (Scheme 1). The acetonide

* Corresponding author.

was removed from the fully protected lactone **9**, prepared from **4** as previously described,[6] by treatment with aqueous acetic acid to give the diol **10** (89% yield) which, with *tert*-butyldimethylsilyl chloride in dimethyformamide in the presence of imidazole selectively formed **11** with only the hydroxyl function at C-5 unprotected (93% yield). Esterification of the free hydroxyl group in **11** with triflic (trifluoromethanesulfonic) anhydride in dichloromethane in the presence of pyridine at 0°C afforded the stable triflate **12** (96% yield).



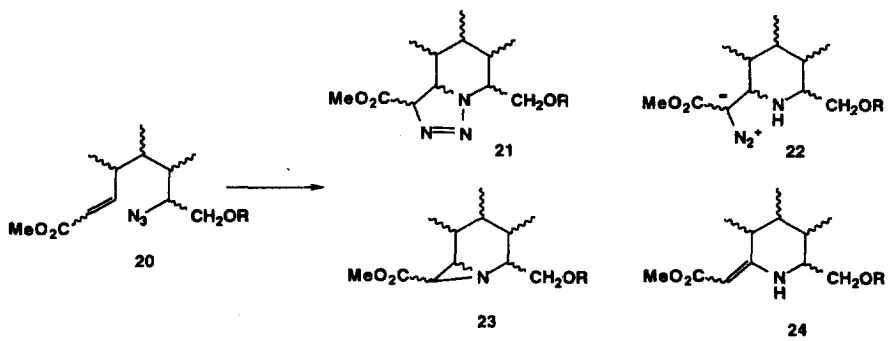
Scheme 1. (i) aq. AcOH, 60°C, 3 h, 89%; (ii) *tert*-$BuMe_2SiCl$, imidazole, DMF, 1 h, 93%; (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0°C, 96%; (iv) $CsOCOCF_3$, butanone; then K_2CO_3, MeOH, 52%; (v) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0°C; then NaN_3, DMF, 90%; (vi) DIBAL-H, THF, 0°C, 81%; (vii) toluene, 120°C, 6 h, 54%; (viii) Br_2, CH_3CCl_3, 83%; (ix) aq. CF_3COOH, quant.; (x) NaOH, H_2O; then Amberlite IR-120, H^+ form, 87%.

Attempts to displace the triflate in **12** with trifluoroacetate using a sodium counterion gave low yields of inverted product. However, caesium trifluoroacetate in butanone afforded, after methanolysis of the intermediate trifluoroacetate ester, the L-*idono*-lactone **13** (52% yield). There are many cases where moderate to good yields of inverted alcohols may be formed with caesium — as opposed to sodium — trifluoroacetate as the oxygen nucleophile for the S_N2 displacement; also, the initially formed trifluoroacetate ester can be readily ester exchanged with methanol without affecting the integrity of the lactone ring.[7] The overall yield of the protected L-*idono*-lactone **13** from the D-*glucono*-lactone **9** is 42%; the sequence may be carried out to produce several grams of product.

Esterification of the L-*idono*-lactone **13** with triflic anhydride, followed by reaction of the resulting triflate with sodium azide in dimethylformamide gave the D-*gluco*-azide **3** (90% yield). Reduction of

the azidolactone **3** with diisobutylaluminum hydride (DIBAL-H) in tetrahydrofuran at 0°C gave the lactols **15** (89% yield), together with a small amount of the diol **14** (11% yield). Hemiacetal **15**, *via* its open chain form, in toluene reacted with the stabilised ylid **16** to form initially a mixture of the esters **2** which on further heating underwent an intramolecular [1,3]-dipolar cycloaddition to give a triazoline **17** as a single isolated product in 52% yield.

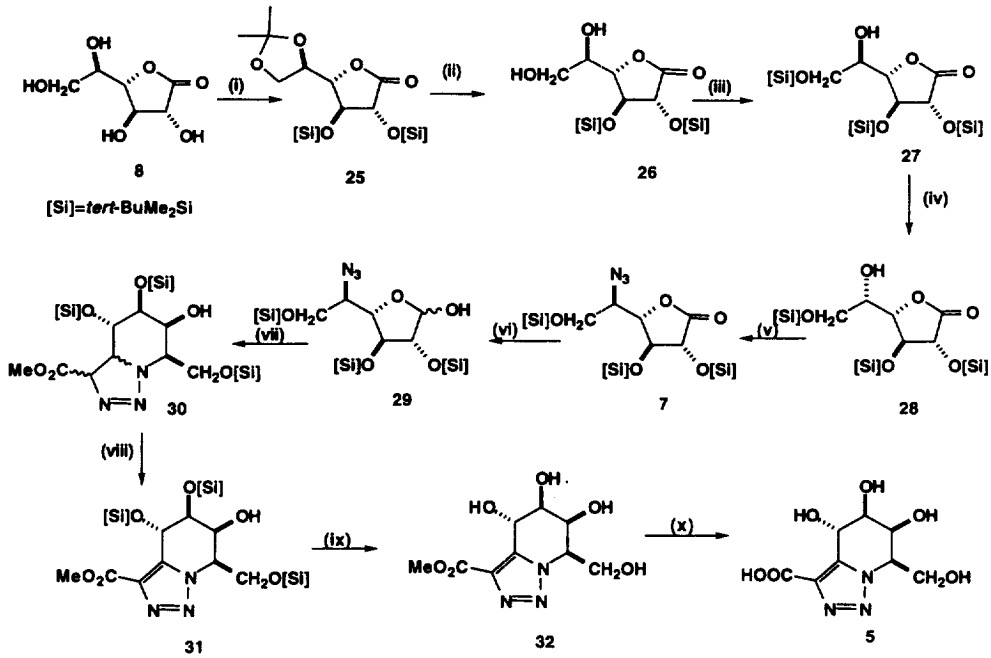
The intramolecular dipolar cycloaddition of azides with α,β-unsaturated esters **20** initially gives triazolines **21** but these may subsequently decompose by a number of different pathways[8,9] to give diazoesters **22**, vinylogous urethane **24** or aziridine[10] carboxylic esters **23**[11] (Scheme 2). The factors which determine which products are formed depend on a number of variables, including temperature, solvent, and in particular the stereochemistry of the carbohydrate moiety present; attempts are in progress to allow control of each different pathway, since a number of novel sugar carbohydrate analogues might all be prepared from a single precursor. Nonetheless, on this occasion, a single stable triazoline **17** was isolated; the infra-red spectrum of **17** indicates the absence of a C=C, a diazoalkane functionality or an azide; the carbonyl stretch at 1744 cm^{-1} showed the presence of a saturated ester. The ^1H and ^{13}C NMR spectra of **17** also support the structure proposed, including six doublets in the range δ 53–78 in the ^{13}C NMR spectrum. The relative configurations at C-3 and C-3a of the triazoline **17** could not be readily determined with certainty, but these stereogenic centres are lost in the next step. All attempts to deprotect the triazoline **17** were unsuccessful, leading to substantial decomposition to very polar products.



Scheme 2. Intramolecular [1,3]-Dipolar cycloaddition of 6-azido-α,β-unsaturated esters.

Oxidation of the triazoline **17** with bromine in trichloroethane in the presence of sodium acetate (to neutralise the HBr produced) gave the triazole methyl ester **18** in 83% yield; in the ^{13}C NMR spectrum, the signals for C-3 and C-3a were singlets at δ 136.6 and 137.6 with only four doublets in the range δ 65–70. The silyl protecting groups in **18** were removed in quantitative yield in aqueous trifluoroacetic acid to afford **19**. Subsequent hydrolysis of the methyl ester **19** with aqueous sodium hydroxide followed by purification with ion exchange resin gave the target D-*gluco*-triazole carboxylic acid **1** in 87% yield.

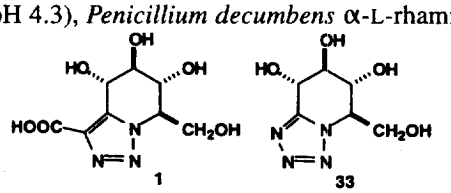
The D-*galacto*-triazole carboxylic acid **5** was prepared by an analogous route from D-*galactono*-lactone **8**. Minor modification of the literature procedure[12] gave the fully protected lactone **25** from which the acetonide was removed by acid hydrolysis to afford the diol **26** in 62% overall yield from **8**. Protection of the primary hydroxyl function in **26** as the *tert*-butyldimethylsilyl ether gave **27** (97% yield). Subsequent triflation of the remaining hydroxyl group in **27**, followed by sequential treatment with caesium trifluoroacetate and basic methanol produced the protected L-*altrono*-lactone **28** (63% yield from **27**). Further reaction of **29** with triflic anhydride, followed by reaction of the resulting triflate with sodium azide in dimethylformamide, afforded the D-*galacto*-azidolactone **7** in 82% yield. This sequence allows the preparation of 10 g amounts of **7** in an overall yield of 31% from D-*galactono*-lactone **8** (Scheme 3).



Scheme 3. (i) $CuSO_4$, Me_2CO; then *tert*-$BuMe_2SiCl$, DMF, 60°C; (ii) aq. AcOH, 60°C, 3 h, 62% over 3 steps; (iii) *tert*-$BuMe_2SiCl$, imidazole, DMF, 1 h, 97%; (iv) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0°C; then $CsOCOCF_3$, butanone; then K_2CO_3, MeOH, 63%; (v) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0°C; then NaN_3, DMF, 82%; (vi) DIBAL-H, THF, 0°C, 78%; (vii) $Ph_3P{=}CHCOOMe$, toluene, 120°C, 2 h, 58%; (viii) Br_2, CH_3CCl_3, 68%; (ix) aq. CF_3COOH, 93%; (x) NaOH, H_2O; then Amberlite IR-120, H^+ form, 97%.

Reduction of the azidolactone **7** with DIBAL-H afforded the azidolactols **29** (78% yield) which underwent a Wittig reaction in its open chain form with the stabilised ylid **16** followed by an intramolecular [1,3]-dipolar cycloaddition to give a single triazoline **30**, the stereochemistry of which at the new stereogenic centres was not firmly established, in 58% yield. As in the case of the D-*gluco*-isomer **17**, the D-*galacto*-triazoline **30** was efficiently oxidised by bromine to the corresponding triazole **31** (68% yield). The silyl groups in **31** were removed by acid hydrolysis to give the methyl ester **32** (93% yield), which was subsequently hydrolysed with aqueous sodium hydroxide to produce the target triazole carboxylic acid **5** (97% yield).

The effects of the triazole acids **1** and **5**, and their corresponding methyl esters **19** and **32**, on a number of glycosidases were investigated; the enzymes had a wide range of pH optima, so that both the carboxylic acids themselves and the corresponding carboxylate anions have been studied as potential inhibitors. No significant inhibition of the following glycosidases at their optimum pH (as indicated in brackets) was observed by either of the triazole carboxylic acids or the methyl esters at over 500 μM for yeast α-glucosidase (pH 4.0), rice α-glucosidase (pH 6.5), almond β-glucosidase (pH 5.0), green coffee bean α-galactosidase (pH 6.5), *E. coli* β-galactosidase (pH 7.3), bovine liver β-galactosidase (pH 7.3), Jack bean α-mannosidase (pH 4.5), human placenta α-fucosidase (pH 5.6), bovine kidney β-N-acetylglucosaminidase (pH 4.3), *Penicillium decumbens* α-L-rhamnosidase (pH 4.0).[13]



The D-*gluco*-triazole carboxylic acid **1** was also tested as a potential inhibitor of glycogen

phosphorylase b (GPb) as part of a long term project in identifying new targets for the treatment of late-onset diabetes;[14] The results obtained at pH 6.8 at which the acid **1** would be completely ionised as the corresponding carboxylate ion were compared with those obtained for the D-*gluco*-tetrazole **33**.[15] Lineweaver–Burk plots of the kinetic data obtained for the inhibition of GPb by triazole carboxylate **1** with respect to phosphate, assayed in the direction of glycogen breakdown, showed that the carboxylate acts as a competitive inhibitor of the enzyme with respect to phosphate, with a K_i value of 7.4 mM. Thus the carboxylate **1** is 140 times less effective than nojirimycin tetrazole analogue **33** (K_i=0.053 mM). In contrast, the tetrazole **33** shows uncompetitive inhibition, binds at the catalytic site and promotes the binding of substrate phosphate through direct interactions as shown by kinetic and X-ray diffraction experiments. Modelling studies indicate that in order for the triazole carboxylate to bind in a position similar to that of nojirimycin tetrazole, it would require conformational shifts of the side chains of catalytic site amino acid residues, such as Asn284 and Thr378, from the positions observed in the ternary GPb-tetrazole-phosphate complex structure.

In summary, this paper describes the synthesis of triazoles fused to the anomeric and ring nitrogen position of analogues of D-*gluco*- and D-*galacto*-pyranoses, and a preliminary investigation into some of their biological properties.

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 FTIR spectrophotometer. Mass spectra were recorded on a VG Masslab 20-250, BIO-Q by desorption chemical ionisation (DCI NH_3), chemical ionisation (CI NH_3), electrospray or thermospray, or atmospheric pressure chemical ionisation ($APCI^+$ or $APCI^-$) as stated. High resolution mass spectra (HRMS) were recorded on a VG Autospec mass spectrometer using chemical ionisation. Exact mass measurement (EMM) was performed on a Prototype Micromass LCT mass spectrometer. Samples were measured by negative ion ESI relative to PEG-Diacid as internal standard. 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 plastic or 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 2 M 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-*Glucono*-lactone **4** was converted to the fully protected derivative **9** as previously described.[6]

Glucotriazole carboxylic acid 1

*2,3-Di-O-*tert*-butyldimethylsilyl*-D-glucono-*1,4-lactone 10*

A solution of the acetonide **9** (6.17 g, 13.8 mmol) in aqueous acetic acid (60 ml, 80% in water) was heated at 60°C for 3 h when t.l.c. (ethyl acetate:hexane=1:10) showed no starting material (R_f 0.9) and mainly one product (R_f 0.1). The solvents were removed under reduced pressure, coevaporated with toluene; the residue was purified by flash chromatography to afford the diol **10** (5 g, 89%) as a white solid, m.p. 72–73°C $[\alpha]_D^{21}$ +45.0 (c 0.33, chloroform); ν_{max} (film) 1790 cm^{-1} (C=O), 3436 cm^{-1} (OH); m/z APCI(+) 247 (100%), 407 ($M+H^+$, 52%); $\delta_H(CDCl_3)$: 0.17 (12 H, app s, $SiMe_2$), 0.93 (18 H, app s, $SiCMe_3$), 1.96 (1 H, bs, OH-6), 2.68 (1 H, d, J=5.4 Hz, OH-5), 3.83–3.81 (1 H, m,

H-6), 3.93–3.91 (1 H, m, H-6′), 4.04–4.01 (1 H, m, H-5), 4.12 (1 H, d, $J_{2,3}$=2.9 Hz, H-2), 4.34 (1 H, t, J~3.5 Hz, H-3), 4.54 (1 H, dd, $J_{4,5}$=8.9, $J_{4,3}$=4.1 Hz, H-4); δ_C($CDCl_3$): −5.1, −4.9, −4.8, −4.6 (4 q, 2 $SiMe_2$,), 17.8, 18.0 (2 s, 2 Si*C*Me_3), 25.5, 25.6 (2 q, 2 Si*C*Me_3), 63.7 (t, C-6), 68.2, 74.5, 74.9, 80.8 (4 d, C-2, C-3, C-4, C-5), 174.3 (s, C-1). Found: C 53.25, H 9.54; $C_{18}H_{38}O_6Si_2$ requires: C 53.16, H 9.42%.

*2,3,6-Tri-O-*tert*-butyldimethylsilyl-*D-glucono-*1,4-lactone 11*

A solution of the diol **10** (5 g, 12 mmol) in dry dimethylformamide (40 ml) with imidazole (2.5 g, 36 mmol) and *tert*-butyldimethylsilyl chloride (2 g, 13 mmol) was stirred at room temperature for 1 h, at which time t.l.c. (ethyl acetate:hexane=1:10) showed no starting material (R_f 0) and one product (R_f 0.7). Buffer solution (pH 7) was added and the mixture was extracted with dichloromethane, washed with brine (4×100 ml), dried (magnesium sulfate), filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography to give the trisilyl ether **11** (5.9 g, 93%) as a white solid, m.p. 56–58°C; $[\alpha]_D^{21}$ +35.4 (c 0.28, chloroform); ν_{max} (film) 1790 cm^{-1} (C=O); m/z (APCI+) 521 ($M+H^+$, 100%); δ_H ($CDCl_3$): 0.17, 0.16, 0.15, 0.14, 0.10 (18 H, 5 s, $SiMe_2$), 0.91, 0.90 (27 H, 2 s, $SiCMe_3$), 2.54 (1 H, d, J=7.0 Hz, OH), 3.80 (1 H, dd, $J_{5,6}$=2.3, $J_{6,6'}$=10.3 Hz, H-6), 3.87 (1 H, dd, $J_{5,6'}$=3.2 Hz, H-6′), 3.95–3.91 (1 H, m, H-5), 4.00 (1 H, d, $J_{2,3}$=1.2 Hz, H-2), 4.22 (1 H, dd, $J_{3,4}$=3.0 Hz, H-3), 4.51 (1 H, dd, $J_{4,5}$=9.2 Hz, H-4); δ_C ($CDCl_3$): −8.7, −6.1, −5.6, −5.5, −5.3, −5.2, −5.0 (7 q, 3 $SiMe_2$,), 17.2, 17.8, 18.1 (3 s, 3 Si*C*Me_3), 25.4, 25.5, 25.7 (3 q, 3 Si*C*Me_3), 63.8 (t, C-6), 67.2, 74.3, 75.1, 80.8 (4 d, C-2, C-3, C-4, C-5), 173.8 (s, C-1). Found: C 55.52, H 10.35; $C_{24}H_{52}O_6Si_3$ requires: C 55.34, H 10.06%.

*2,3,6-Tri-O-*tert*-butyldimethylsilyl-5-O-trifluoromethanesulfonyl-*D-glucono-*1,4-lactone 12*

A solution of the trisilyl ether **11** (5.7 g, 11 mmol) in dry dichloromethane (30 ml) was cooled to 0°C under nitrogen. Dry pyridine (2.2 ml, 27 mmol) and trifluoromethanesulfonic anhydride (2.4 ml, 14 mmol) were added and the solution was stirred for 10 min at which time t.l.c. (ethyl acetate:hexane=1:10) showed no starting material (R_f 0.7) and one product (R_f 0.8). The reaction mixture was diluted with dichloromethane, washed with buffer solution (pH 7) and then brine. The organic layer was dried (magnesium sulfate), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography to afford the triflate **12** (6.8 g, 96%) as a white solid, m.p. 92–93°C; $[\alpha]_D^{21}$ +31.3 (c 0.34, chloroform); ν_{max} (film) 1797 cm^{-1} (C=O); m/z (CI NH_4^+) 670 ($M+NH_4^+$, 72%); δ_H ($CDCl_3$): 0.08, 0.09, 0.16, 0.18 (18 H, 4 s, 3 $SiMe_2$), 0.89, 0.90, 0.91 (27 H, 3 s, $SiCMe_3$), 3.97 (1 H, dd, $J_{5,6}$=5.4, $J_{6,6'}$=12.4 Hz, H-6), 4.03 (1 H, dd, $J_{5,6'}$=2.6 Hz, H-6′), 4.10 (1 H, d, $J_{2,3}$=2.4 Hz, H-2), 4.30 (1 H, dd, $J_{3,4}$=4.0 Hz, H-3), 4.95 (1 H, dd, $J_{4,5}$=4.9 Hz, H-4), 5.20 (1 H, app dt, H-5); δ_C ($CDCl_3$): −6.0, −5.4, −5.3, −5.1, −4.8 (5 q, 3 $SiMe_2$), 17.5, 17.8, 17.9 (3 s, 3 Si*C*Me_3), 25.3, 25.4 (2 q, 3 Si*C*Me_3), 61.1 (t, C-6), 74.2, 74.6, 79.5, 85.9 (4 d, C-2, C-3, C-4, C-5) 118.6 (q, J=327 Hz, CF_3), 172.8 (s, C-1); HRMS found: 670.290344. Calcd for ($M^+NH_4^+$) 670.290835.

*2,3,6-Tri-O-*tert*-butyldimethylsilyl-*L-idono-*1,4-lactone 13*

The triflate **12** (6.8 g, 10 mmol) in dry 2-butanone (45 ml) was treated with caesium trifluoroacetate (7.9 g, 32 mmol) and the reaction mixture was heated at 60°C under nitrogen for 1 h. The solvent was removed under reduced pressure and the residue dissolved in methanol (15 ml) with potassium carbonate (590 mg, 4.2 mmol). The mixture was stirred at room temperature for 30 min, at which time t.l.c. (ethyl acetate:hexane=1:20) showed mainly one product (R_f 0.4). Ethyl acetate (200 ml) was added and the mixture was washed with buffer solution (pH 7, 50 ml) and then brine (50 ml). The organic phase was dried (magnesium sulfate), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography to give the inverted alcohol **13** (2.8 g, 52%) as a white solid, m.p. 42–43°C; $[\alpha]_D^{21}$ +69.4, (c 0.55, chloroform); ν_{max} (film) 1797 cm^{-1} (C=O), 3470 cm^{-1} (OH); m/z (APCI+) 521 ($M+H^+$, 100%), 543 ($M+Na^+$, 45%), δ_H ($CDCl_3$): 0.08, 0.12,

0.15, 0.16, 0.20 (12 H, 5 s, 3 $SiMe_2$), 0.91, 0.92, 0.93 (18 H, 3 s, 3$SiCMe_3$), 2.35 (1 H, d, J=4.0 Hz, OH-5), 3.67 (1 H, dd, $J_{5,6}$=6.0, $J_{6,6'}$=9.8 Hz, H-6), 3.71 (1 H, dd, $J_{5,6'}$=7.0 Hz, H-6'), 4.06–4.02 (1 H, m, H-5), 4.43 (1 H, dd, $J_{3,4}$=8.1, $J_{4,5}$=0.6 Hz, H-4), 4.49 (1 H, app t, J~7.1 Hz, H-3), 4.60 (1 H, d, $J_{2,3}$=7.0 Hz, H-2), δ_C ($CDCl_3$): −5.5, −4.9, −4.8, −4.7, −4.5 (5 q, 3 $SiMe_2$), 17.8, 18.1 (2s, 3 Si*C*Me_3), 25.6, 25.7 (2q, 3 Si*C*Me_3), 62.9 (t, C-6), 69.1, 73.9, 76.2, 77.4 (4 d, C-2, C-3, C-4, C-5), 173.8 (s, C-1). Found: C 55.47, H 10.22; $C_{24}H_{52}O_6Si_3$ requires: C 55.34, H 10.06%.

*5-Azido-2,3,6-tri-O-*tert*-butyldimethylsilyl-5-deoxy-*D-glucono*-1,4-lactone 3*

The *idono*-lactone **13** (2.8 g, 5.3 mmol) in dry dichloromethane (30 ml) at 0°C under nitrogen was treated with dry pyridine (1.1 ml, 13.6 mmol) and triflic anhydride (1.2 ml, 7.1 mmol); after 10 minutes, t.l.c. (ethyl acetate:hexane=1:20) showed no starting material (R_f 0.4) and one product (R_f 0.6). The reaction was quenched with buffer solution (pH 7) then diluted with dichloromethane and washed with aqueous HCl (2 M), buffer solution (pH 7) and brine. The organic layer was dried (magnesium sulfate), filtered and concentrated *in vacuo*. The crude triflate was dissolved in dry dimethylformamide (45 ml) and treated with sodium azide (383 mg, 0.74 mmol). After 3 hours at room temperature, t.l.c. (ethyl acetate:hexane=1:20) showed mainly one product (R_f 0.5). Ethyl acetate and brine were added, the mixture was extracted with ethyl acetate, washed with brine, dried (magnesium sulfate), filtered and the solvent removed under reduced pressure to give, after purification by flash chromatography (ethyl acetate:hexane=1:20) the azidolactone **3** (2.6 g, 90%) as a white solid. m.p. 80–83°C; $[\alpha]_D^{21}$ +17.6 (c 0.17, chloroform); ν_{max} (film) 1793 cm^{-1} (C=O), 2103 cm^{-1} (azide); m/z (APCI+) 518 ($M-N_2+H^+$, 100%), 546 ($M+H^+$, 10%); δ_H ($CDCl_3$): 0.12, 0.14, 0.16, 0.17 (18 H, 4 s, 3 $SiMe_2$), 0,90, 0.91, 0.92 (27 H, 3 s, 3 $SiCMe_3$), 3.73 (1 H, ddd, $J_{5,6'}$=2.4, $J_{5,6}$=6.4, $J_{5,4}$=10.0 Hz, H-5), 3.90 (1 H, dd, $J_{6,6'}$=10.8 Hz, H-6), 3.97 (1 H, d, $J_{2,3}$ <1.0 Hz, H-2), 4.11–4.13 (2 H, m, H-6', H-3), 4.47 (1 H, dd, $J_{3,4}$=2.8 Hz, H-4), δ_C ($CDCl_3$): −5.7, −5.6, −5.4, −5.2, −5.0, −4.7 (6 q, 3 $SiMe_2$), 17.8, 17.9, 18.1 (3s, 3 Si*C*Me_3), 25.4, 25.5, 25.7 (3 q, 3 Si*C*Me_3), 64.1 (t, C-6), 59.1, 74.1, 74.8, 79.8 (4d, C-2, C-3, C-4, C-5), 173.6 (s, C-1). Found: C 53.00, H 9.54, N 7.68; $C_{24}H_{51}N_3O_5Si_3$ requires: C 52.80, H 9.42, N 7.70%.

*5-Azido-2,3,6-tri-O-*tert*-butyldimethylsilyl-5-deoxy-αβ-*D*-glucofuranose* ***15*** *and 5-azido-2,3,6-tri-O-*tert*-butyldimethylsilyl-5-deoxy-*D*-glucitol* ***14***

The azidolactone **3** (334 mg, 0.61 mmol) in tetrahydrofuran (9 ml) at 0°C under nitrogen was treated with diisobutylaluminium hydride (1.5 M in toluene, 0.49 ml, 1.2 equivalents), and the reaction mixture was stirred for 5 minutes when t.l.c. (ethyl acetate:hexane=1:20) showed no starting material (R_f 0.5) and two products (R_f 0.4, 0.2). The reaction was quenched by addition of saturated aqueous ammonium chloride solution and the mixture was extracted with dichloromethane, washed with aqueous sodium tartrate solution (10%) and brine, dried (magnesium sulfate), filtered and the solvent removed under reduced pressure. The residue was purified by flash chromatography to give an anomeric mixture of the lactols **15** (271 mg, 81%) as a colourless oil, ν_{max} (film) 2098, 2130 cm^{-1} (azide), 3535 cm^{-1} (OH); m/z (APCI+) 502 ($M-N_2-H_2O+H^+$, 96%), 520 ($M-N_2+H^+$, 100%); δ_H ($CDCl_3$) one anomer: 0.12–0.23 (18 H, m of s, 3 $SiMe_2$), 0.94–0.97 (27 H, m of s, 3 $SiCMe_3$), 3.55 (1 H, d, J=12.6 Hz, OH), 3.58 (1 H, ddd, $J_{5,6}$=2.5, $J_{5,6'}$=7.6, $J_{4,5}$=8.4 Hz, H-5), 3.84 (1 H, dd, $J_{6,6'}$=10.6 Hz, H-6), 3.93 (1 H, dd, $J_{3,4}$=1.6 Hz, H-4), 4.03–4.01 (1 H, m, H-2), 4.09 (1 H, bs, H-3), 4.13 (1 H, dd, H-6'), 5.00 (1 H, app d, H-1); other anomer: 0.12–0.23 (18 H, m of s, 3 $SiMe_2$), 0.94–0.97 (27 H, m of s, 3 $SiCMe_3$),3.66 (1 H, ddd, $J_{5,6}$=2.5, $J_{5,6'}$=6.8, $J_{5,4}$=9.7 Hz, H-5), 3.70 (1 H, d, J=12.9 Hz, OH), 3.87 (1 H, dd, $J_{2,3}$=0.9, $J_{2,1}$=3.0 Hz, H-2), 3.92 (1 H, dd, $J_{6,6'}$=10.5 Hz, H-6), 4.02–4.01 (2 H, m, H-4, H-3), 4.18 (1 H, dd, H-6'), 5.45 (1 H, dd, H-1); δ_C ($CDCl_3$); both anomers: −5.7 to −4.5 (m of q, 3 $SiMe_2$), 17.8–18.1 (m of s, 3 Si*C*Me_3), 25.5–25.7 (m of q, 3 Si*C*Me_3), 64.9, 65.2 (2 t, C-6), 60.5, 60.8, 76.3, 76.5, 77.7, 79.9, 80.1, (7 d, C-2, C-3, C-4, C-5), 98.2, 104.1 (2 d, C-1). Further elution gave a small amount of the more polar diol **14** (38 mg, 11%); ν_{max} (film) 2098 cm^{-1} (azide), 3415 cm^{-1} (OH);

m/z (APCI+) 550 ($M+H^+$, 100%); δ_H ($CDCl_3$): 0.10, 0.11, 0.18, 0.14, 0.15, 0.16 (18 H, 6 s, 3 $SiMe_2$), 0.92, 0.93 (27 H, 2 s, 3 $SiCMe_3$), 3.22 (1 H, ddd, $J_{5,6}$=6.6, $J_{5,6'}$=2.6, $J_{5,4}$=9.6 Hz, H-5), 3.62 (1 H, dd, $J_{1,2}$=3.8, $J_{1,1'}$=11.7 Hz, H-1), 3.75 (1 H, app dt, $J_{1,2}$ ~$J_{1'2}$=3.9, $J_{2,3}$=5.2 Hz, H-2), 3.78 (1 H, d, H-4), 3.83 (1 H, dd, $J_{1',2}$=4.1 Hz, H-1'), 3.89 (1 H, dd, $J_{6,6'}$=10.7 Hz, H-6), 3.98 (1 H, d, H-3), 4.14 (1 H, dd, H-6'); δ_C($CDCl_3$): −5.6, −4.8, −4.7, −4.3 (4 q, $SiMe_2$), 17.9, 18.2 (2 s, 3 $SiCMe_2$), 25.7 (q, 3 $SiCMe_3$), 61.5, 64.3 (2 t, C-1, C-6), 63.5, 65.9, 71.1, 73.2 (4 d, C-2, C-3, C-4, C-5).

*(3RS,3aRS,4S,5S,6R,7R)-Methyl 4,5-bis(*tert*-butyldimethylsilyloxy)-7-[(*tert*-butyl-dimethylsilyloxy)-methyl]-3,3a,4,5,6,7-hexahydro-6-hydroxy-pyrido[1,2-c][1,2,3]triazole-3-carboxylate* **17**

A solution of the stabilised ylid **16** (199 mg, 0.595 mmol) and the azidolactol **15** (258 mg, 0.471 mmol) in toluene (20 ml) was heated under reflux for 6 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography (hexane:ethyl acetate=10:1) to give the triazoline **17** (152 mg, 54%) as a colourless wax, $[\alpha]_D^{24}$ −191.3 (c 0.61, $CHCl_3$); ν_{max} (film) 3527 cm^{-1} (b, OH), 1744 cm^{-1} (C=O); m/z (APCI+): 604 (100%, $M+H^+$), 576 (60%, $M+H^+-N_2$); δ_H (500 MHz, $CDCl_3$): 0.03, 0.04, 0.12, 0.13, 0.14, 0.16 (18 H, 6 s, 3 $SiMe_2$), 0.88, 0.89, 0.92 (27 H, 3 s, 3 $SiCMe_3$), 3.53 (1 H, d, J=11.3 Hz, OH-6), 3.67 (1 H, m, H-4), 3.82 (1 H, dd, J_1=7.5, J_{gem}=10.3 Hz, 0.5 CH_2), 3.83 (3 H, s, OMe), 3.87 (1 H, m, H-6), 3.91 (1 H, dd, J=7.9 Hz, 0.5 CH_2), 4.00 (1 H, app t, J~3.3 Hz, H-5), 4.22 (1 H, dd, $J_{3a,4}$=2.1, $J_{3,3a}$=9.1 Hz, H-3a), 4.54 (1 H, m, H-7), 4.91 (1 H, d, H-3): δ_C (50.3 MHz, $CDCl_3$): −5.6 to −4.3 (m of q, 3 $SiMe_3$), 17.6, 17.9, 18.0 (3 s, $SiCMe_3$), 25.4–25.7 (m of q, 3 CMe_3), 52.7 (q, OMe), 53.5, 57.2, 67.4, 71.9, 74.3, 77.9 (6 d, C-3, C-3a, C-4, C-5, C-6, C-7) 63.2 (t, CH_2), 169.2 (s, *C*OOMe). Found: C, 53.21; H, 9.74; N, 6.88%; $C_{27}H_{57}N_3O_6Si_3$ requires C, 53.69; H, 9.51; N, 6.96%.

*(4S,5S,6R,7R)-Methyl 4,5-bis(*tert*-butyldimethylsilyloxy)-7-[(*tert*-butyldimethyl silyloxy)methyl]-4,5,6,7-tetrahydro-6-hydroxy-pyrido[1,2-c][1,2,3]triazole-3-carboxylate* **18**

A bromine solution (0.4 ml, 1 M in 1,1,1-trichloroethane) was added to a suspension of the triazoline **17** (150 mg, 0.248 mmol) and sodium acetate (92 mg, 1.12 mmol) in 1,1,1-trichloroethane (15 ml). The reaction was stirred at room temperature for 8 hours and then diluted with ether (100 ml). The reaction mixture was washed successively with sodium thiosulfate solution (10%, 30 ml) and brine (30 ml); the organic layer was dried (magnesium sulfate) and the solvent removed. Flash chromatography (hexane:ethyl acetate=10:1) of the residue yielded the triazole ester **18** (124 mg, 83%) as a colourless solid, m.p. 109–111°C (plates, hexane); $[\alpha]_D^{24}$ −33.5 (c 0.55, $CHCl_3$); ν_{max} (film) 3496 cm^{-1} (b, OH), 1720 cm^{-1} (C=O); m/z (APCI+): 602 (100%, $M+H^+$); δ_H (500 MHz, $CDCl_3$): 0.07, 0.09, 0.11, 0.13, 0.14, 0.33 (18 H, 6 s, 3 $SiMe_2$), 0.79, 0.85, 0.92 (27 H, 3 s, 3 $SiCMe_3$), 3.80 (1 H, app t, J~10.1 Hz, 0.5 CH_2), 3.97 (3 H, s, OMe), 4.27 (1 H, d, J=10.6 Hz, OH-6), 4.35–4.38 (2 H, m, H-5, H-7), 4.56 (1 H, m, H-6), 4.84 (1 H, dd, J_1=5.7, J_{gem}=10.4 Hz, 0.5 CH_2), 5.40 (1 H, dd, J_1=1.6, J_2=3.1 Hz, H-4); δ_C (50.3 MHz, $CDCl_3$): −5.6 to −5.2 (m of q, 3 $SiMe_2$), 17.8–17.9 (m of s, 3 $SiCMe_3$), 25.4–25.6 (m of q, 3 $SiCMe_3$), 52.0 (q, OMe), 63.8 (t, CH_2), 65.5, 65.7, 68.4, 69.9 (4 d, C-4, C-5, C-6, C-7), 136.6, 137.6 (2 s, C-3, C-3a), 161.8 (*C*OOMe). Found: C, 53.80; H, 9.46; N, 6.79%; $C_{27}H_{55}N_3O_6Si_3$ requires C, 53.87; H, 9.21; N, 6.98%.

(4S,5S,6R,7R)-Methyl 4,5,6,7-tetrahydro-4,5,6-tris(hydroxy)-7-[(hydroxy)methyl]-pyrido[1,2-c][1,2,3]-triazole-3-carboxylate **19**

The silyl triazole **18** (170 mg, 0.282 mmol) was dissolved in aqueous trifluoroacetic acid (50%, 10 ml) and allowed to stand for 18 h at room temperature. After concentration *in vacuo* the residue was purified by flash chromatography (chloroform:methanol=9:1) to give the methyl ester **19** (73 mg, quant.) as a colourless oil, $[\alpha]_D^{23}$ −84.5 (c 0.48, MeOH); ν_{max} (film) 3370 cm^{-1} (b, OH), 1724 cm^{-1} (C=O); m/z (APCI+): 260 (100%, $M+H^+$); δ_H (500 MHz, MeOH-*d*4): 3.90 (1 H, dd, $J_{4,5}$=6.9, $J_{5,6}$=8.7 Hz, H-5), 3.97 (3 H, s, OMe), 4.11 (1 H, dd, $J_{6,7}$=7.7 Hz, H-4), 4.15 (1 H, dd, J_1=2.6, J_{gem}=11.9 Hz,

0.5 CH_2), 4.38 (1 H, app dt, H-7), 4.51 (1 H, dd, J=3.4 Hz, 0.5 CH_2), 4.89 (1 H, d, H-4); δ_C (50.3 MHz, MeOH-*d*4): 53.1 (q, OMe), 59.7 (t, CH_2), 65.6, 67.3, 68.5, 75.0 (4 d, C-4, C-5, C-6, C-7), 137.4, 142.5 (2 s, C-3, C-3a), 163.9 (s, *C*OOMe).

(4S,5S,6R,7R)-4,5,6,7-Tetrahydro-4,5,6-tris(hydroxy)-7-[(hydroxy)methyl]-pyrido[1,2-c][1,2,3]-triazole-3-carboxylic acid ***1***

Aqueous sodium hydroxide (0.40 ml, 0.5 M in water) was added to a solution of the methyl ester **19** (51 mg, 0.196 mmol) in water (10 ml). After 12 h at room temperature, the clear solution was passed down a small column of ion exchange resin (3 ml, Amberlite IR-120, H^+ form). Concentration and freeze drying of the eluate gave the triazole carboxylic acid **1** (42 mg, 87%) as lyophilisate, $[\alpha]_D^{23}$ −96.8 (c 0.25, H_2O); ν_{max} (film) 3392 cm^{-1} (b, OH), 1716 cm^{-1} (C=O); m/z (electrospray): 244 (100%, $M-H^+$); δ_H (250 MHz, D_2O): 3.92 (1 H, dd, $J_{4,5}$=7.8, $J_{5,6}$=9.9 Hz, H-5), 4.05 (1 H, dd, $J_{6,7}$=8.6 Hz, H-6), 4.18 (1 H, dd, J_1=2.1, J_{gem}=12.7 Hz, 0.5 CH_2), 4.43 (1 H, m, H-7), 4.58 (1 H, dd, J=2.4 Hz, 0.5 CH_2), 4.96 (1 H, d, H-4); δ_C (50.3 MHz, D_2O): 58.2 (t, CH_2), 64.3, 66.4, 67.2, 74.6 (4 d, C-4, C-5, C-6, C-7), 137.7, 141.4 (2 s, C-3, C-3a), 164.8 (s, *C*OOMe); EMM (ESI) found 244.0570, $M-H^+$ requires 244.0564.

Galactotriazole carboxylic acid ***5***

*2,3-Di-O-*tert*-butyldimethylsilyl-*D-galactono-*1,4-lactone* ***26***

D-*Galactono*-1,4-lactone **8** (20 g, 0.111 mol) and copper(II) sulfate (40 g, anhydrous) were suspended in acetone (1 l, AR grade) and vigorously stirred for 12 hours at room temperature. The mixture was filtered through Celite and the filtrate was stirred for 1 hour with sodium hydrogencarbonate (1.0 g). After further filtration, the solution was concentrated to give the crude monoacetonide (24.8 g) which was dissolved in dimethylformamide (80 ml) and treated with *tert*-butyldimethylsilyl chloride (48.7 g, 0.323 mol and imidazole (55.1 g, 0.809 mol) at 60°C for 4 hours under nitrogen. The solution was then allowed to cool before diluting with ether (600 ml) and washing with water (2×100 ml) and brine (100 ml). Drying of the organic phase over magnesium sulfate and concentration gave the fully protected lactone **25** as a pale yellow solid (58 g), which can be used without any further purification in the next step, $[\alpha]_D^{22}$ −6.3 (c 1.44, $CHCl_3$); ν_{max} (KBr) 1800 cm^{-1} (C=O); m/z (APCI+): 464 (100%, $M+NH_4^+$), 447 (21%, $M+H^+$); δ_H (500 MHz, $CDCl_3$): 0.15, 0.16, 0.22 (12 H, 3 s, 2 $SiMe_2$), 0.92, 0.96, (18 H, 2 s, 2 $SiCMe_3$), 1.39, 1.41, (6 H, 2 s, CMe_2), 3.95 (1 H, dd, $J_{4,5}$=1.9, $J_{4,3}$=7.2 Hz, H-4), 3.98 (1 H, dd, $J_{6',6}$=7.8 Hz, H-6′), 4.10 (1 H, dd, $J_{6,5}$=6.9, $J_{6,6'}$=8.1 Hz, H-6), 4.27 (1 H, ddd, $J_{5,4}$=1.9, $J_{5,6}$=7.3, $J_{5,6'}$=8.9 Hz, H-5), 4.35 (1 H, d, $J_{2,3}$=8.1 Hz, H-2), 4.40 (1 H, app t, J=8.0 Hz, H-3′), δ_C (50.3 MHz, $CDCl_3$): −5.1, −4.9, −4.6, −4.3, (4 q, 2 $SiMe_2$), 17.6, 18.1 (2 s, 2 $SiCMe_3$), 25.5, 25.6 (2 q, CMe_2) 25.9 (q, 2 $SiCMe_3$), 65.1 (t, C-6), 72.2, 75.3, 76.2, 78.4 (4 d, C-2, C-3, C-4, C-5), 110.1 (s, CMe_2), 172.8 (s, C-1). Found: C, 56.48; H, 9.84%; $C_{21}H_{42}O_6Si_2$ requires C, 56.46; H, 9.47%.

A suspension of the fully protected lactone **25** (58 g, crude) in aqueous acetic acid (80%, 1 l) was warmed to 60°C with stirring. After 3 hours the resulting solution was allowed to cool to ambient temperature and the solvent was then removed under high vacuum. The residue was co-evaporated with toluene (3×300 ml) to remove traces of acetic acid. Flash column chromatography (hexane:ethyl acetate=1.5:1) of the residue (48 g) afforded the diol **26** (28.3 g, 62% over three steps) as a clear oil, $[\alpha]_D^{22}$ −5.7 (c 1.21, $CHCl_3$); ν_{max} (film) 1797 cm^{-1} (C=O); m/z (APCI+): 407 (100%, $M+H^+$); δ_H (500 MHz, $CDCl_3$): 0.15, 0.16, 0.21 (12 H, 3 s, 2 $SiMe_2$), 0.91, 0.94 (18 H, 2 s, 2 $SiCMe_3$), 3.75 (1 H, m, H-5), 3.84–3.81 (2 H, m, H-6, H-6′), 4.09 (1 H, dd, $J_{4,5}$=1.2, $J_{4,3}$=7.3 Hz, H-4), 4.35 (1 H, d, $J_{2,3}$=7.6 Hz, H-2), 4.46 (1 H, app t, J=7.5 Hz, H-3); δ_C (50.3 MHz, $CDCl_3$): −4.8, −4.5, −4.1 (3 q, 2 $SiMe_2$), 17.7, 18.1 (2 s, 2 $SiCMe_3$), 26.6, 25.7 (2 q, 2 $SiCMe_3$), 63.8 (t, C-6), 68.1, 74.3, 75.9, 80.9 (4 d, C-2, C-3, C-4, C-5), 173.6 (s, C-1). Found: C, 53.07; H, 9.81%; $C_{18}H_{38}O_6Si_2$ requires C, 53.16; H, 9.42%.

2,3,6-Tri-O-tert-*butyldimethylsilyl*-D-galactono-*1,4-lactone 27*

The diol **26** (12.9 g, 31.8 mmol), imidazole (5.9 g, 87.5 mmol) and *tert*-butyldimethylsilyl chloride (5.3 g, 35 mmol) were dissolved in the minimum of dimethylformamide (20 ml) at 0°C under N_2. The solution was then allowed to warm to ambient temperature and stirred for 1 hour, diluted with ether (300 ml), washed with brine (2×50 ml) and dried (magnesium sulfate). After concentration, the residue was purified by flash chromatography (hexane:ethyl acetate=19:1) to give the trisilylated lactone **27** (16.1 g, 97%) as a white solid, m.p. 77–78°C (hexane); $[\alpha]_D^{22}$ −13.5 (c 1.12, $CHCl_3$); ν_{max} (film) 1792 cm^{-1} (C=O); m/z (APCI+): 521 (100%, M+H$^+$); δ_H (500 MHz, $CDCl_3$): 0.08, 0.14, 0.15, 0.16, 0.22 (18 H, 5 s, 3 $SiMe_2$), 0.90, 0.91, 0.95 (27 H, 3 s, 3 $SiCMe_3$), 2.16 (1 H, d, $J_{5\text{-}OH,5}$=6.9 Hz, 5-OH), 3.75 (1 H, app t, J=6.5 Hz, H-6), 3.78 (1 H, m, H-5), 4.15 (1 H, dd, $J_{4,3}$=7.3, $J_{4,5}$=0.9 Hz, H-4), 4.36 (1 H, d, $J_{2,3}$=7.7 Hz, H-2), 4.45 (1 H, app t, J=7.5 Hz, H-3); δ_C (50.3 MHz, $CDCl_3$): −5.5, −4.8, −4.4, −4.1 (4 q, 3 $SiMe_2$), 17.7, 18.2 (2 s, 3 $SiCMe_3$), 25.6, 25.7, 25.8 (3 q, 3 $SiCMe_3$), 63.3 (t, C-6), 68.3, 74.4, 76.1, 79.6 (4 d, C-2, C-3, C-4, C-5), 173.0 (s, C-1). Found: C, 55.06; H, 10.51%; $C_{24}H_{52}O_6Si_3$ requires C, 55.33; H, 10.06%.

2,3,6-Tri-O-tert-*butyldimethylsilyl*-L-altrono-*1,4-lactone 28*

Triflic anhydride (7.0 ml, 41.6 mmol) was added to a solution of the lactone **27** (18.1 g, 34.7 mmol) in dichloromethane (100 ml) and pyridine (7 ml, 86.7 mmol) at 0°C under an atmosphere of nitrogen. After stirring for 15 min, the reaction was quenched with water (0.3 ml) and the solution was passed down a silica plug (eluent: dichloromethane). Concentration *in vacuo* afforded the crude triflate, which was redissolved in butanone (70 ml). The solution was treated with caesium trifluoroacetate (25.6 g, 104 mmol) and was stirred for 1 h at 60°C, allowed to cool to room temperature and, after addition of methanol (250 ml), stirred for a further 12 h. After removal of the solvent the residue was partitioned between ether (500 ml) and water (50 ml). The ether layer was separated, washed with brine (100 ml), dried (magnesium sulfate) and then concentrated. Purification by flash chromatography (ethyl acetate:hexane=19:1) gave the inverted alcohol **28** (11.3 g, 63%). m.p. 53–55°C (hexane); $[\alpha]_D^{22}$ +22.0 (c 0.97, $CHCl_3$); ν_{max} (film) 1798 cm^{-1} (C=O); m/z (APCI+): 521 (100%, M+H$^+$); δ_H (500 MHz, $CDCl_3$): 0.10, 0.13, 0.14, 0.18, 0.19 (18 H, 5 s, 3 $SiMe_2$); 0.90, 0.91, 0.92 (27 H, 3 s, 3 $SiCMe_3$), 2.59 (1 H, d, $J_{5\text{-}OH,5}$=6.4 Hz, 5-OH), 3.79–3.73 (3H, m, H-5, H-6, H-6′), 4.09 (1 H, d, $J_{2,3}$=2.7 Hz, H-2), 4.19 (1 H, dd, $J_{4,3}$=2.4, $J_{4,5}$=8.0 Hz, H-4), 4.38 (1 H, app t, J=2.6 Hz, H-3), δ_C (50.3 MHz, $CDCl_3$): −5.7, −5.6, −4.9, −4.7 (4 q, 3 $SiMe_2$) 17.7, 17.9, 18.0 (3 s, $SiCMe_3$), 25.5, 25.7(2 q, 3 $SiCMe_3$), 62.7 (t, C-6), 70.7, 75.5, 75.8, 84.8,(4 d, C-2, C-3, C-4, C-5), 174.6 (s, C-1). Found: C, 55.25; H, 10.29%; $C_{24}H_{52}O_6Si_3$ requires C, 55.33; H, 10.06%.

5-Azido-2,3,6-tri-O-tert-*butyldimethylsilyl-5-deoxy*-D-galactono-*1,4-lactone 7*

Triflic anhydride (3.9 ml, 23.2 mmol) was added to a solution of lactone **28** (10.0 g, 19.2 mmol) in dichloromethane (75 ml) and pyridine (5 ml, 61.9 mmol) at 0°C under an atmosphere of nitrogen. After stirring for 15 min the reaction was quenched with water (0.2 ml) and the solution was passed down a silica plug (eluent: dichloromethane). Concentration *in vacuo* afforded the crude triflate, which was redissolved in dimethylformamide (50 ml) and treated with sodium azide (1.53 g, 23.5 mmol). After stirring for 2.5 hours at room temperature the solution was added to a mixture of water (200 ml) and brine (200 ml). Extraction with ether (4×100 ml), washing of the combined extracts with brine (100 ml), drying over magnesium sulfate and evaporation gave an oily residue which was purified by flash chromatography (hexane:ethyl acetate=19:1) to afford the azidolactone **7** (8.6 g, 82%) as a colourless oil, $[\alpha]_D^{23}$ −26.6 (c 0.58, $CHCl_3$); ν_{max} (film) 2105 cm^{-1} (azide), 1807 cm^{-1} (C=O); m/z (APCI+): 518 (100%, M+H$^+$−N_2) 546 (5%, M+H$^+$); δ_H (500 MHz, $CDCl_3$): 0.11, 0.12, 0.16, 0.17, 0.22 (18 H, 5 s, 3 $SiMe_2$), 0.91, 0.92, 0.95 (27 H, 3 s, 3 $SiCMe_3$), 3.60 (1 H, ddd, H-5), 3.89 (1 H, dd, $J_{5,6}$=6.1, $J_{6,6'}$=10.2 Hz, H-6), 3.97 (1 H, dd, $J_{5,6'}$=7.5 Hz, H-6′), 4.11 (1 H, dd, $J_{4,5}$=1.9, $J_{3,4}$=7.4 Hz, H-4), 4.34 (1 H, d, $J_{2,3}$=8.0 Hz, H-2), 4.40 (1 H, app t, H-3); δ_C (50.3 MHz, $CDCl_3$): −5.6, −5.5,

−4.7, −4.4, −4.0 (5 q, 3 $SiMe_2$), 17.7, 18.2 (2 s, 3 $SiCMe_3$), 25.6, 25.7 (2 q, 3 $SiCMe_3$), 60.3 (d, C-5), 62.8 (t, C-6), 75.2, 75.8, 78.3 (3 d, C-2, C-3, C-4), 172 (s, C-1). Found: C, 52.63; H, 9.72; N, 7.89%; $C_{24}H_{51}N_3O_5Si_3$ requires C, 52.80; H, 9.42; N, 7.70%.

*5-Azido-2,3,6-tri-O-*tert*-butyldimethylsilyl-5-deoxy-αβ-*D*-galactofuranose* ***29***

Diisobutylaluminium hydride (5.3 ml, 1.5 M sol. in toluene) was added to a solution of the azidolactone **7** (1.73 g, 3.17 mmol) in tetrahydrofuran (50 ml) at 0°C under an atmosphere of nitrogen. After stirring for 30 min, the reaction mixture was quenched with saturated ammonium chloride solution (0.5 ml) and diluted with dichloromethane (200 ml). The organic layer was washed with potassium sodium tartrate solution (3×50 ml, 10%) and brine (50 ml) and dried (magnesium sulfate). Concentration and purification by flash chromatography afforded the azidolactols **29** (1.35 g, 78%) as a colourless oil, ν_{max} (film) 2100 cm^{-1} (azide); m/z (APCI+): 520 (15%, $M+H^+-N_2$); δ_H (500 MHz, $CDCl_3$): minor anomer: 0.10–0.16 (18 H, m, 3 $SiMe_3$), 0.89, 0.92, 0.95 (27 H, 3 s, 3 $SiCMe_3$), 3.42 (1 H, m, H-5), 3.43 (1 H, d, J=10.5 Hz, OH), 3.79 (1 H, app t, J=4.1 Hz, H-4), 3.85 (2 H, m, H-6, H-6′), 3.92 (1 H, app t, J=3.9 Hz, H-2), 4.15 (1 H, app t, J=4.0 Hz, H-3), 5.24 (1 H, dd, $J_{1,2}$=3.9 Hz, H-1), major anomer: 0.10–0.14 (18 H, m, 3 $SiMe_2$), 0.90, 0.91, 0.92 (27 H, 3 s, 3 $SiMe_3$), 3.40 (1 H, d, J=10.3 Hz, OH), 3.56 (1 H, app dt, H-5), 3.72 (1 H, dd, $J_{5,6}$=6.5, $J_{6,6'}$=10.8 Hz, H-6), 3.81 (1 H, dd, $J_{5,6'}$=4.4 Hz, H-6′), 4.00, 4.06 (2 H, 2 m, H-2, H-3), 4.20 (1 H, dd, $J_{4,5}$=6.9, $J_{3,4}$=2.3 Hz, H-4), 5.15 (1 H, app, d, H-1); δ_C (50.3 MHz, $CDCl_3$): −5.5 to −4.3 (m, 3 $SiMe_2$ major and minor), 17.7–18.3 (m, 3 $SiCMe_3$ major and minor), 25.6–25.8 (m, 3 $SiCMe_3$ major and minor), 62.9, 77.4, 78.4, 81.8 (4 d, C-2, C-3, C-4, C-5 minor), 63.3 (t, C-6 minor), 63.5 (t, C-6 major), 64.0, 78.7, 81.8, 86.1 (4 d, C-2, C-3, C-4, C-5 major), 97.1 (d, C-1 minor), 103.6 (d, C-1 major). Found: C, 52.47; H, 10.07; N, 7.97%; $C_{24}H_{53}N_3O_5Si_3$ requires C, 52.61; H, 9.76; N, 7.69%.

*(3RS,3aRS,4S,5S,6S,7R)-Methyl 4,5-bis(*tert*-butyldimethylsilyloxy)-7-[(*tert*-butyl-dimethylsilyloxy)-methyl]-3,3a,4,5,6,7-hexahydro-6-hydroxy-pyrido[1,2-c][1,2,3]triazole-3-carboxylate* ***30***

A solution of the stabilised ylid **16** (616 mg, 1.84 mmol) and the azidolactol **29** (840 mg, 1.53 mmol) in toluene (20 ml) was stirred at 120°C for 2 h. The reaction mixture was then concentrated and the residue purified by flash chromatography (hexane:ethyl acetate=10:1–8:1) to give a single triazoline **30** (540 mg, 58%) as a colourless solid, m.p. 172–174°C (needles, hexane); $[\alpha]_D^{23}$ −248.9 (c 0.18, $CHCl_3$); ν_{max} (film) 3271 cm^{-1} (b, OH), 1741 cm^{-1} (C=O); m/z (APCI+): 604 (100%, $M+H^+$), 576 (25%, $M+H^+-N_2$); δ_H (500 MHz, benzene-*d*6): −0.05 to 0.19 (18 H, m of s, 3 $SiMe_2$), 0.77, 0.87, 0.88 (27 H, 3 s, 3 $SiCMe_3$), 3.01 (1 H, d, J=7.1 Hz, OH-6), 3.24 (3 H, s, COOMe), 3.71 (1 H, dd, $J_{3a,4}$=2.3, $J_{4,5}$=4.0 Hz, H-4), 3.99 (1 H, app t, H-5), 4.01 (1 H, dd, J_1=7.2, J_{gem}=10.3 Hz, 0.5 CH_2), 4.24 (1 H, app dt, H-6), 4.41 (1 H, dd, J_1=7.5, J_{gem}=10.3 Hz, 0.5 CH_2), 4.46 (1 H, dd, $J_{3,3a}$=7.5 Hz, H-3a), 4.76 (1 H, app q, H-7), 5.16 (1 H, d, H-3); δ_C (50.3 MHz, $CDCl_3$): −5.6 to −4.2 (6 q, 3 $SiMe_3$), 14.1, 17.7, 18.0 (3 s, $SiCMe_3$), 25.5, 25.7, 25.72 (3 q, 3 CMe_3), 52.8 (q, OMe), 53.6, 56.8, 67.5, 72.0, 74.4, 78.2 (6 d, C-3, C-3a, C-4, C-5, C-6, C-7) 63.5 (t, CH_2), 169.3 (s, *C*OOMe); HRMS (CI+) found 604.3622, $M+H^+$ requires: 604.3633.

*(4S,5S,6S,7R)-Methyl 4,5-bis(*tert*-butyldimethylsilyloxy)-7-[(*tert*-butyldimethyl silyloxy)methyl]-4,5,6,7-tetrahydro-6-hydroxy-pyrido[1,2-c][1,2,3]triazole-3-carboxylate* ***31***

A bromine solution (0.3 ml, 1 M in 1,1,1-trichloroethane) was added to a suspension of the galactotriazoline **30** (109 mg, 0.180 mmol) and sodium acetate (61 mg, 0.742 mmol) in 1,1,1-trichloroethane (10 ml). The reaction was stirred at room temperature for 3 h before being diluted with ether (100 ml). After successive washing with sodium thiosulfate solution (10%, 30 ml) and brine (30 ml), the organic layer was dried (magnesium sulfate) and concentrated. Flash chromatography (hexane:ethyl acetate=10:1) of the residue yielded the protected triazole **31** (73 mg, 68%) as a solid, m.p. 161–163°C (hexane); $[\alpha]_D^{24}$ −13.2 (c 0.35, $CHCl_3$); ν_{max} (film) 3350 cm^{-1} (OH), 1732 cm^{-1}

(C=O); m/z (APCI+): 602 (100%, M+H$^+$); δ_H (500 MHz, $CDCl_3$): 0.06, 0.13, 0.16, 0.18, 0.21, 0.27 (18 H, 6 S, 3 $SiMe_2$), 0.83, 0.84, 0.96 (27 H, 3 s, 3 $SiCMe_3$), 3.97 (3 H, s, COOMe), 4.25 (1 H, app t, J~2.8 Hz, H-5), 4.39 (1 H, dd, J_1=5.1, J_{gem}=9.7 Hz, 0.5 CH_2), 4.49 (1 H, app t, J~9.9 Hz, 0.5 CH_2), 4.70 (1 H, dd, $J_{5,6}$=2.1, $J_{6,7}$=6.5 Hz, H-6), 5.04 (1 H, ddd, H-7), 5.30 (1 H, d, $J_{4,5}$=3.3 Hz, H-4); δ_C (50.3 MHz, $CDCl_3$): −5.8, −5.4, −5.3, −5.1, −5.0, −4.8 (6 q, 3 $SiMe_2$), 17.7 (3 s, 3 $SiCMe_3$), 25.3–25.5 (3 q, 3 $SiCMe_3$), 52.0 (q, *OMe*), 57.1, 64.3, 66.9, 74.8 (4 d, C-4, C-5, C-6, C-7), 66.1 (t, CH_2), 136.2, 138.8 (2 s, C-3, C-3a), 161.5 (s, *C*OOMe). Found: C, 53.73; H, 9.46; N, 6.81%; $C_{27}H_{55}N_3O_6Si_3$ requires C, 53.87; H, 9.21; N, 6.98%.

(4S,5S,6S,7R)-Methyl 4,5,6,7-tetrahydro-4,5,6-tris(hydroxy)-7-[(hydroxy)methyl]-pyrido[1,2-c][1,2,3]-triazole-3-carboxylate **32**

The tri-O-silylated-triazole **31** (105 mg, 0.174 mmol) was dissolved in aqueous trifluoroacetic acid (50%, 10 ml) and the solution was stirred for 18 hours at room temperature. After concentration *in vacuo* the residue was purified by flash chromatography (chloroform:methanol=9:1) to give the deprotected methyl ester **32** (42 mg, 93%) as a colourless solid, m.p. 104–106°C ($CHCl_3$/MeOH); $[\alpha]_D^{23}$ −41.3 (c 0.72, MeOH); ν_{max} (film) 3390 cm^{-1} (b, OH), 1722 cm^{-1} (C=O); m/z (APCI+): 260 (82%, M+H$^+$); δ_H (500 MHz, MeOH-*d*4): 3.96 (3 H, s, OMe), 4.01 (1 H, dd, $J_{5,6}$=2.3, $J_{4,5}$=6.4 Hz, H-5), 4.13 (1 H, dd, J_1=6.3, J_{gem}=11.4 Hz, 0.5 CH_2), 4.40 (1 H, dd, J=4.2 Hz, 0.5 CH_2), 4.55 (1 H, dd, $J_{6,7}$=4.0 Hz, H-6), 4.70 (1 H, app dt, H-7), 5.10 (1 H, d, H-4); δ_C (50.3 MHz, MeOH-*d*4): 52.9 (q, OMe), 60.2 (t, CH_2), 63.1, 66.1, 69.1, 74.2 (4 d, C-4, C-5, C-6, C-7), 137.2, 142.0 (2 s, C-3, C-3a), 163.6 (s, *C*OOMe).

(4S,5S,6S,7R)-4,5,6,7-Tetrahydro-4,5,6-tris(hydroxy)-7-[(hydroxy)methyl]-pyrido[1,2-c][1,2,3]-triazole-3-carboxylic acid **5**

Aqueous sodium hydroxide (0.27 ml, 0.5 M in water) was added to a solution of methyl ester **32** (35 mg, 0.135 mmol) in water (5 ml). After stirring for 12 h at room temperature, the clear solution was passed down a small column with ion exchange resin (3 ml, Amberlite IR-120, H$^+$ form). Concentration and freeze drying gave the D-galactotriazole carboxylic acid **5** (32 mg, 97%) as lyophilisate, $[\alpha]_D^{23}$ −50.0 (c 0.37, H_2O); ν_{max} (KBr) 3392 cm^{-1} (b, OH), 1599 cm^{-1} (C=O); m/z (electrospray): 244 (100%, M−H$^+$); δ_H (500 MHz, D_2O): 3.98 (1 H, dd, $J_{5,6}$=2.4, $J_{4,5}$=7.8 Hz, H-5), 3.99 (1 H, dd, J_1=6.3, J_{gem}=11.9 Hz, 0.5 CH_2), 4.29 (1 H, dd, J=5.3 Hz, 0.5 CH_2), 4.43 (1 H, app t, J~2.6 Hz, H-6), 4.62 (1 H, app dt, H-7), 5.00 (1 H, d, H-4); δ_C (50.3 MHz, D_2O): 60.1 (t, CH_2), 62.4, 65.4, 69.7, 73.3 (4 d, C-4, C-5, C-6, C-7), 137.5, 140.7 (2 s, C-3, C-3a), 164.7 (s, *C*OOH); EMM (ESI) found 244.0570, M−H$^+$ requires 244.0570.

Materials and methods and assays for glycogen phosphorylase b (GPb)

AMP, NADP, Glucose-6-P dehydrogenase, glucose-1,6-diphosphate, glycogen, and other chemicals were obtained from Sigma Chemical Co. Oyster glycogen was freed of AMP by the method of Helmreich and Cori.[16] Rabbit phosphoglucomutase purchased from Sigma as a suspension in 2.5 M ammonium sulfate was dialyzed against 60 mM β-glycerophosphate, 4.5 mM EDTA, 3 mM DTT buffer (pH 6.8) just before use. GPb was isolated from rabbit skeletal muscle according to Fischer and Krebs,[17] using 2-mercaptoethanol (recrystallized at least four times) instead of L-cysteine. Bound nucleotides were removed from the enzyme as previously described.[18] Protein concentration was determined from absorbance measurments at 280 nm using an absorbance index A1% 1cm=1.32.[19]

Kinetic studies

Phosphorylase activity in the direction of glycogen breakdown was carried out using the auxiliary assay system as described by Helmreich and Cori[16] with some modifications. The final reaction mixtures were 0.3 ml and contained 1.2 μg GPb, 4 μg glucose-6-phosphate dehydrogenase, 10 μg phosphoglucomutase, 1 mM NADP, 0.001 mM glucose-1,6-diphosphate, 20 mM imidazole, 2.4 mM

β-glycerophosphate, 10 mM 2-mercaptoethanol, 0.3 mM EDTA, 0.1 mM DTT, 10 mM magnesium acetate, 1 mM AMP, 0.5% glycogen and a range of concentrations of phosphate and inhibitor (pH 6.8 and 30°C). Enzyme, AMP and glycogen were preincubated for 15 min at 30°C, before the reaction was initiated by this mixture. The reaction was stopped by addition of sodium dodecyl sulfate to reach a final concentration of 0.1%, at times selected in order to secure linearity of the assay. NADPH formed in the reaction was measured at 340 nm. A molar extinction coefficient of 6220 M^{-1} cm^{-1} for NADPH at 340 nm was used in the calculations. Data were analyzed by the use of the nonlinear regression program Grafit[20] as previously described.[21]

Acknowledgements

This work was supported by EPSRC post doctoral fellowships, and by Greek GSRT (PENED-225) to NGO. We wish to acknowledge the assistance of the final year project students S. Avlonites and D. Katharios in the kinetic experiments.

References

1. P. Ermert, A. Vasella, *Helv. Chim. Acta*, **1991**, *74*, 2043; P. Ermert, A. Vasella, M. Weber, K. Rupitz, S. G. Withers, *Carbohydr. Res*, **1993**, *250*, 113; T. D. Heightman, P. Ermert, D. Klein, A. Vasella, *Helv. Chim. Acta*, **1995**, *78*, 514; B. Davis, T. W. Brandstetter, C. Smith, L. Hackett, B. G. Winchester, G. W. J. Fleet, *Tetrahedron Lett.*, **1995**, *36*, 7507; T. W. Brandstetter, B. Davis, D. Hyett, C. Smith, L. Hackett, B. G. Winchester, G. W. J. Fleet, *Tetrahedron Lett.*, **1995**, *36*, 7511.
2. K. Tatsuta, Y. Ikeda, S. Miura, *J. Antibiot.*, **1996**, *49*, 836.
3. A. Frankwoski, C. Seliga, D. Bur, J. Streith, *Helv. Chim. Acta*, **1991**, *74*, 934; K. Tatsuta, S. Miura, S. Ohta, H. Gunjii, *J. Antibiot.*, **1995**, *48*, 286; T. Aoyagi, H. Suda, K. Uotani, F. Kojima, T. Aoyama, K. Horiguchi, M. Hamada, T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1404; T. Aoyama, H. Naganawa, H. Suda, K. Uotani, T. Aoyagi, T. Takeuchi, *J. Antibiot.*, 1992, **45**, 1557; D. H. Hua, F. Q. Zhang, J. S. Chen, P. D. Robinson, *J. Org. Chem.*, 1994, **59**, 5084.
4. T. D. Heightman, M. Locatelli, A. Vasella, *Helv. Chim. Acta*, **1996**, *79*, 2190.
5. T. Granier, N. Panday, A. Vasella, *Helv. Chim. Acta*, **1997**, *80*, 97; T. Granier, F. Gaiser, L. Hintermann, A. Vasella, *Helv. Chim. Acta*, **1997**, *80*, 1443.
6. R. E. Lee, M. D. Smith, R. J. Nash, R. C. Griffiths, M. McNeil, R. K. Grewal, W. Yan, G. S. Besra, P. J. Brennan, G. W. J. Fleet, *Tetrahedron Lett.*, **1997**, *38*, 6733.
7. A. A. Bell, L. Pickering, M. Finn, C. de la Fuente, T. M. Krülle, B. G. Davis, G. W. J. Fleet, *Synlett*, **1997**, 1077.
8. J. G. Buchanan, A. R. Edgar, B. D. Hewitt, *J. Chem. Soc., Perkin Trans. 1*, **1987**, 2371.
9. C. Herdeis, T. Schiffer, *Tetrahedron*, **1996**, *52*, 14745.
10. B. Bernet, A. R. C. B. Murty, A. Vasella, *Helv. Chim. Acta*, **1990**, *73*, 940.
11. L. Pickering, G. W. J. Fleet, in preparation.
12. G. W. J. Fleet, J. C. Son, *Tetrahedron*, **1988**, *44*, 2637.
13. For details of the glycosidase assays, see A. A. Watson, R. J. Nash, M. R. Wormald, D. J. Harvey, S. Dealler, E. Lees, N. Asano, H. Kizu, R. C. Griffiths, A. J. Cairns, G. W. J. Fleet, *Phytochemistry*, **1997**, *46*, 255.
14. Y. Blériot, K. H. Smelt, J. Cadefau, M. Bollen, W. Stalmans, K. Biggadike, L. N. Johnson, N. G. Oikonomakos, A. L. Lane, S. Crook, D. J. Watkin, G. W. J. Fleet, *Tetrahedron Lett.*, **1996**, *37*, 7155; T. M. Krülle, C. de la Fuente, K. A. Watson, M. Gregoriou, L. N. Johnson, K. E. Tsitsanou, S. E. Zographos, N. G. Oikonomakos, G. W. J. Fleet, *Synlett*, **1997**, 211; C. de la Fuente, T. M. Krülle, K. A. Watson, M. Gregoriou, L. N. Johnson, K. E. Tsitsanou, S. E. Zographos, N. G. Oikonomakos, G. W. J. Fleet, *Synlett*, **1997**, 485 and references therein.
15. E. P. Mitchell, S. G. Withers, P. Ermert, A. T. Vasella, E. F. Garman, N. G. Oikonomakos, L. N. Johnson, *Biochemistry*, **1996**, *35*, 7341.
16. E. J. M. Helmreich, C. F. Cori, *Proc. Natl Acad. Sci. USA*, **1964**, *51*, 131.

17. E. H. Fischer, E. G. Krebs, *Methods Enzymol.*, **1962**, *5*, 369.
18. A. E. Melpidou, N. G. Oikonomakos, *FEBS Lett.*, **1983**, *154*, 105.
19. L. L. Kastenschmidt, J. Kastenschmidt, E. Helmreich, *Biochemistry*, **1968**, *7*, 3590.
20. R. J. Leatherbarrow, GraFit Version 3.0., Staines, UK: Erithakus Software, 1992.
21. N. G. Oikonomakos, M. Kontou, S. E. Zographos, K. A. Watson, L. N. Johnson, C. J. F. Bichard, G. W. J. Fleet, K. R. Acharya, *Protein Sci.*, **1995**, *4*, 2469.

(Received in UK 3 October 1997)