Synthesis of 1,2[^{3}H]-1,2-Epoxy Analogue of Fructose-6P, an Affinity Label of *Escherichia coli* Glucosamine-6P Synthase

Caroline LERICHE§, Loïc RENÉ§, Florence DEROUET¶, Bernard ROUSSEAU¶ & Bernard BADET§

§ *Institut de Chimie des Substances Naturelles-CNRS UPR 2301, 91198 Gif-sur-Yvette, France*
¶ *Service des Molécules Marquées-CEA., Département de Biologie Cellulaire et Moléculaire, CEA-Saclay, 91191 Gif-sur-Yvette, France*

Summary

1,2-anhydroglucitol-6P, a known inhibitor of glucose-6P isomerase, behaved as a fructose-6P site-directed irreversible inhibitor of bacterial glucosamine-6P synthase. The lack of reproducibility of the aldolase-mediated condensation of dihydroxyacetone phosphate and glycidaldehyde followed by borohydride reduction previously described prompted us to develop a chemical route to this compound and its radiolabelled counterpart. The compound was synthesized in 13 steps from D-arabinose with a 6% overall yield. Tritium introduction was performed at step 11 (**3** → **4**) allowing isolation of the title compound of high specific radioactivity.

Keywords: 1,2-anhydroglucitol-6P, glucosamine-6P synthase, affinity label.

Introduction

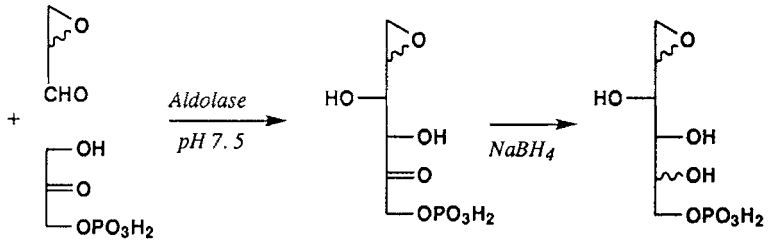
Glucosamine-6P synthase (L-glutamine:D-fructose-6P amidotransferase, GlmS) catalyzes the conversion of fructose-6P to glucosamine-6P using the amide function of L-glutamine as nitrogen donor [1]. This transformation and the 1R stereospecificity demonstrated in proton abstraction [2] are typical features of 1R-keto/aldose isomerase class [3]. A member of this class, glucose-6P isomerase, which catalyzes the interconversion of fructose-6P and glucose-6P, is inactivated by an analogue of fructose-6P, 1,2-anhydroglucitol-6P **6**. We therefore investigated the behavior of this compound towards bacterial GlmS.

CCC 0362–4803/95/111115–07

Received 15 June 1995
Revised 16 June 1995

Results

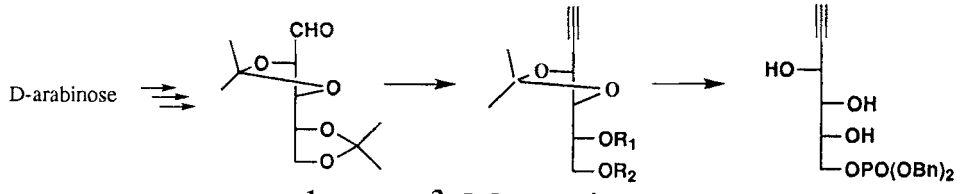
1,2-anhydrohexitols-6P were first obtained (Scheme 1) by condensation of commercially available dihydroxy acetone phosphate with racemic glycidaldehyde [4] catalyzed by rabbit muscle aldolase [5].



Scheme 1: chemoenzymatic synthesis of 1,2-anhydrohexitol-6P

Sodium borohydride reduction of the keto group gave 1,2-anhydrohexitol-6P which irreversibly inhibited (K_{irr}= 1. 5 mM, $k_{inact.}$= 0. 6 min^{-1}) GlmS. However the difficulties encountered in the reproducibility of the reduction step prompted us to develop a more efficient method to produce the inhibitor. Based on the specificity of the enzyme for its substrate we choosed configuration D for C_5, and devised then a synthetic route using D-arabinose as starting material.

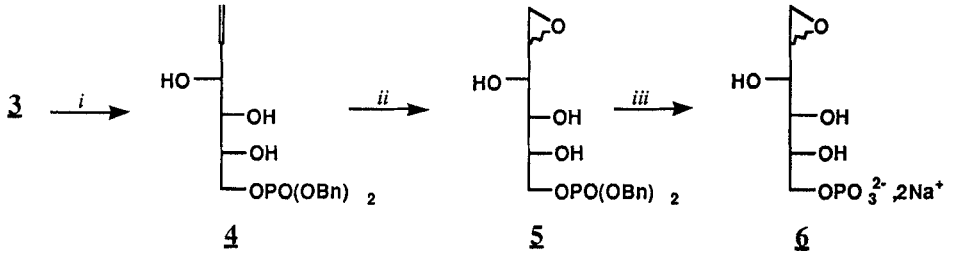
2,3:4,5-di-*O*-isopropylidene-aldehydo-D-arabinose **1** (Scheme 2) was synthesized in 51% yield from D-arabinose as described for the L isomer [6]. One carbon homologation to the acetylenic compound **2a** was optimized to 72% yield under the very mild conditions using dibenzyl-(1-diazo-2-oxopropyl)-phosphonate in methanol in the presence of potassium carbonate [7]. Selective hydrolysis of 5,6-isopropylidene protection with aqueous acetic acetic [8] afforded **2b** in 80% yield. The extreme phosphorylating ability of *N,N*-diisopropyl-dibenzyl phosphoramidite [9] required the protection of the secondary C_5-hydroxyl group as tetrahydropyranyl ether which was accomplished in an overall 75% via the selectively protected silyl derivative **2d**. Compound **2e** was then phosphorylated in 86% yield and deprotected in 25% aqueous trifluoroacetic acid to afford 6-dibenzylphosphate-1,2-dideoxy-D-arabino-hex-1-ynitol **3** in 78% yield.



1 **2**a R_1-R_2= $C(Me)_2$; b R_1= R_2= H
c R_1= H, R_2= *t*BDPS; d R_1= THP, R_2= *t*BDPS
e R_1= THP, R_2= H; f R_1= THP, R_2= $PO(OBn)_2$ **3**

Scheme 2: synthesis of phosphorylated compound **3**

Partial hydrogenation (Scheme 3) at room temperature and under atmopsheric pressure using quinoleine-poisonned Lindlar catalyst gave quantitatively **4** contaminated by only 10% of fully reduced derivative **4a**. *Meta*-chloroperbenzoic acid epoxidation of the mixture followed by HPLC purification afforded **5** in 70% yield as a colorless solid. Deprotection of the phosphate group was completed following a 10 min hydrogenation in methanol in the presence of Pd/C (10%). The sensitivity of the epoxide to phosphate acidic moiety required the use of 1.25 equivalent of triethylamine in the reaction mixture. After removal of the catalyst, the phosphate ester must be treated with a stoichiometric amount of bicarbonate before evaporation to dryness. Determination of epoxide (60%) in dilute aqueous solution was performed using Ross method [10].



Scheme 3: synthesis of **6** (*i*: H_2/Lindlar/AcOEt-quinolein, *ii*: *m*CPBA/CH_2Cl_2, *iii*: H_2/Pd-C/MeOH,Et_3N then $HNaCO_3$)

A similar sequence was performed for the synthesis of radioactive derivative. After hydrogenation under tritium gas (10 Ci), the side product **4a** was removed by HPLC to afford 1,2-[^{3}H]-**4** (S.A: 59 Ci/mmol). This compound (50 mCi) was diluted 250 fold with non radioactive compound to perform epoxidation. The epoxidation product was purified by flash chromatography to afford **5** with a specific radioactivity high enough for enzyme labelling (15 mCi/mmol). The identification of labelled residue which requires GlmS inactivation with [^{3}H]-**6**, proteolytic cleavage and radioactive peptide mapping will be reported later.

Experimental

General: ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a 300 MHz Brucker instrument. Chemical shifts δ are expressed in ppm relative to tetramethyl silane. Mass spectra were obtained from a Ribergmag RG1010 with the Chemical Ionisation (CI) method with isobutan or from a Kratos MS 80 for Fast Atom Bombardment (FAB) analysis. Melting points determined with a Kofler block. TLC were performed on Merck-Schuchart 60F254 precoated silica gel (0.2 mm on aluminium). Elemental analyses were performed by Service de Microanalyses, ICSN/CNRS, Gif-sur-Yvette, France.

Abbreviations: *m*CPBA: *meta*-chloroperbenzoic acid, DMF: dimethylformamide, THF: tetrahydrofuran.

1,2-dideoxy-3,4:5,6-di-*O*-isopropylidene-D-arabino-hex-1-ynitol (2a). To a solution of **1** (500 mg, 2.2 mmol) and acetyldimethyldiazophosphonate (637 mg, 3.3 mmol) in ice-cold methanol (15 ml) K_2CO_3 (594 mg, 4;3 mmol) was added in one portion. After 30 min at 0°C and 3 hours at room temperature the reaction mixture was hydrolyzed with sat. NH_4Cl (15 ml) and extracted with ether (3 x 30 ml). The organic extracts were dried over $MgSO_4$, filtered, evaporated and purified by flash chromatography (2:8 ether/pentane as eluent) to afford **2a** (360 mg, 1.46 mmol, 72%) as an oil.

TLC Rf 0.42 (4/6 EtOAc/Heptane); MS M+1=227, M-C_3H_6O = 169; ^{1}H NMR δ 1.33-1.49 (4s, 12H, CH_3-C), 2.56 (d, 1H, H_1), 3.9-4.05 (m, 4H, H_4, H_5, H_6, $H_{6'}$), 4.65 (m, 1H, H_3); ^{13}C NMR δ 25.65, 26.56, 27.24, 27.5 (4 CH_3), 67.29 (C_6), 68.58 (C_3), 74.99 (C_1), 76.7 (C_5), 77.10 (C_2), 82.8 (C_4), 110.5-111.7 (2C(Me)$_2$); Anal. calcd for $C_{12}H_{18}O_4$: C 63.70, H 8.02, O 28,28 found C 63.64 H 8.06 O 28.26.

1,2-dideoxy-3,4-*O*-isopropylidene-D-arabino-hex-1-ynitol (2b). **2a** (3.27 g, 14.5 mmol) was dissolved in 25% aqueous acetic acid (300 ml) and the mixture was stirred for 2 hours at 55°C. After evaporation of the solvent, sat. NaCl (100 ml) was added and the solution was extracted with ethyl acetate (3 x 60 ml). The organic phases were neutralized with sat. HNaCO3 and dried over $MgSO_4$. After filtration, the solvent was removed under vacuum to afford **2b** (1.83 g, 9.8 mmol, 70%) as a colorless solid.

m.p.: 76°C; TLC Rf 0.4 (EtOAc); MS M+1=187, M-C_3H_6O = 129; ^{1}H NMR δ 1.4 (d, 6H, CH_3-C), 2.5 (d, 1H, H_1), 3.6 (m, 2H, H_6), 3.8 (m, 1H, H_4), 4.1 (m, 1H, H_5), 4.6 (dd, 1H, H_3); ^{13}C NMR δ 25.9, 26.9 (2 CH_3), 63.2 (C_6), 71.6 (C_2), 74.8(C_4), 81.6 (C_1), 82.1 (C_5), 110.9 (C(Me)$_2$); Anal. calcd for $C_9H_{14}O_4$: C 58.05, H 7.58, O 34.37 found C 58.09 H 7.62 O 34.54.

1,2-dideoxy-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-D-arabino-hex-1-ynitol (2c). To a mixture of **2b** (100 mg, 0.54 mmol), imidazole (80.9 mg, 2.2 eq.) in dimethylformamide (1.5 ml) *tert*-butyl chlorodiphenyl silane (163.3 mg, 1.1 eq.) was added under argon. After 6 hours at room temperature, DMF was removed under vacuum and the residue was diluted in ethyl acetate (10 ml), washed with water (10 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic phases were dried over $MgSO_4$, filtered and evaporated under vacuum. The crude product was purified by flash chromatography (4:6 EtOAc/Heptane as eluent) to afford **2c** (200 mg, 0.47 mmol, 88%) as a colorless oil.

TLC Rf 0.4 (4:6 EtOAc/Heptane); MS M+1=425, M-C_3H_6O = 367; ^{1}H NMR δ 1.1 (s, 9H, CH_3-CSi), 1.4 (s, 3H, CH_3-C), 1.5 (s, 3H, CH_3-C), 2.6 (d, 1H, H_1), 3.8 (m, 2H, H_6), 4.2 (m, 2H, H_4 & H_5), 4.7 (dd, 1H, H_3), 7.4 (m, 5H, Ar), 7.65 (m, 5H, Ar); ^{13}C NMR δ 25.9-26.9 (5 CH_3), 60.4 (C*t*Bu), 64.6 (C_6), 67.6 (C_3), 72.3 (C_2), 74.3 (C_4), 81.5 (C_1), 82.1 (C_5), 110.9 (C(Me)$_2$), 135,6-127,8 (m, Ar).

1,2-dideoxy-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-5-*O*-tetrahydropyranyl-D-arabino-hex-1-ynitol (2d). Dihydropyrane (1.39g, 5 eq.) and *p*-toluene sulfonic acid (5.7 mg, 0.01 eq.) were added to **2c** (1.38 g, 3.3 mmol) dissolved in CH_2Cl_2 (14 ml) at 0°C. After stirring 10 min at 0°C and 15 min at room temperature, ether (15 ml) was added and the solution was washed with sat. $HNaCO_3$, brine and dried over $MgSO_4$. After evaporation of the solvent the residue was purified by flash chromatography (4:6 EtOAc/Hepatne as eluent) to give **2d** (1.68g, 3.17 mmol, 96%) as colorless gummy solid.

TLC Rf 0.55 (1:1 EtOAc/Heptane); MS M+1=509, M-C_3H_6O= 451, M-THP+1= 425; ^{1}H NMR δ 1.1 (m, 9H, tBu), 1.4-1.8 (m, 12H, CH_3-C + CH_2 of THP), 2.45 (d, 1H, H_1), 3.45 (m, 2H, OCH_2), 3.65-4.1 (m, 4H, H_4, H_5, H_6, $H_{6'}$), 4.2 (m, 1H, O-CH-O-), 4.8 (m, 1H, H_3), 7.25 (m, 5H, Ar), 7.65 (m, 5H, Ar); ^{13}C NMR δ 25.7-26.3 (CH_2), 27.1, 27.3 (2 CH_3), 30.7-31.3 (CH2), 62.3-64 (O-CH_2), diast.), 63,9-64,1 (C_6, diast.), 65.9-67.9 (C_3, diast.), 74.3-74.6 (C_1,diast.), 75.8-76.9 (C_4, diast.), 82.3-82.4 (C_5, diast.), 97.9-99.9 (O-CH-O, diast.), 110.3 ($C(Me)_2$), 111.1 (C*t*Bu), 127.9-129.9-130-136.1 (m, Ar); Anal. calcd for $C_{30}H_{40}O_5Si$: C 70.83, H 7.93, Si 5.52 found C 71.01 H 7.84 Si 4.7.

1,2-dideoxy-3,4-*O*-isopropylidene-5-*O*-tetrahydropyranyl-D-arabino-hex-1-ynitol (2e). 1M tetrabutyl ammonium fluoride (1.07ml, 3.63 mmol) was added to **2d** (1.68 g, 3.3 mmol) in THF (18 ml). After 4 hours stirring at room temperature the solvent was evaporated and the residue was purified by flash chromatography (1:1 EtOAc/heptane as eluent) to give **2e** (790 mg, 2.9 mmol, 88%) as a colorless oil.

TLC Rf 0.2 (1:1 EtOAc/Heptane); MS M+1=271, M-THP+1= 187; ^{1}H NMR δ 1.4 (s, 3H, CH_3), 1.5 (s, 3H, CH_3), 1.5-1.9 (m, 6H, 3CH_2 from THP), 2.5 (d, 1H, H_1), 3.5 (m, 2H, OCH_2), 3.8 (m, 2H, H_6, $H_{6'}$), 4 (m, 1H, H_5), 4.3 (t, 1H, H_4), 4.75 (dd, 1H, H_3), 4.8 (m, 1H, O-CH-O-); ^{13}C NMR δ 25.6-26.5 (2 CH_3), 19,1-20,4-24,5-24,8-30.2-30.6 (3CH2, diast.), 60.9-64.5 (O-CH_2, diast.), 62.3 (C_6), 67.9 (C_3), 73.7 (C_2), 75.5 (C1), 80.6 (C_4), 81.7 (C5), 110 ($C(Me)_2$), 96.5-101.5 (O-CH-O, diast.).

1,2-dideoxy-3,4-*O*-isopropylidene-5-*O*-tetrahydropyranyl-D-arabino-hex-1-ynitol Dibenzyl 6-phosphate (2f). Carefully dried **2e** (790 mg, 2.9 mmol) was added to neat dibenzyl-*N*-*N*-diisopropylphosphoramidite and tetrazole (406 mg, 5.8 mmol) in acetonitrile (3 ml) under Ar atmospher. After 30 min at room temperature, the solution was cooled to -40°C, *m*CPBA (1 g, 5.8 mmol) in CH_2Cl_2 (10 ml) was added and the solution was stirred for 15 min. The mixture was quenched by addition of sodium thiosulfate (1 ml of 1M solution) and the solution was neutralized by sat. $HNaCO_3$ and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over $MgSO_4$, filtered and evaporated. Flash chromatography purification (8:1 EtOAc/Heptane as eluent) afforded **2f** (1.33 g, 2.5 mmol, 86%) as colorless oil.

TLC Rf 0.6 (1:1 EtOAc/Heptane); MS M+1=531, M-THP= 447; ^{1}H NMR δ 1.4 (s, 3H, CH_3), 1.5 (s, 3H, CH_3), 1.4-1.8 (m, 6H, 3CH_2 from THP), 2.5 (d, 1H, H_1), 3.6 (m, 2H, OCH_2), 4.22 (t, 1H, H_4), 4.3 (m, 1H, H_5), 4.8 (m, 2H, O-CH-O- & H_3), 5.2 (m, 4H, CH_2 Bn), 7.4 (s, 10H, Ar); ^{13}C NMR δ 27.7 (2 CH_3), 20.1-26.2-31.4 (3 CH2), 63.3-63.7 (O-CH_2, diast.), 67-68 (C_6, diast.), 75.1 (C_4), 75.9 (C3), 81.1 (C_1), 81.9 (C5), 98.1 ($C(Me)_2$), 101.1 (O-CH-O), 128.6-129.6 (m, Ar).

1,2-dideoxy-D-arabino-hex-1-ynitol Dibenzyl 6-phosphate (3). 2f (500 mg, 0.94 mmol) was dissolved in 25% aqueous trifluoroacetic acid (8 ml) at 0°C. After 30 min stirring at 0°C the solution was poured in 50 ml water and extracted with ethyl acetate. The combined organic phases (50 ml) were neutralized, dried over $MgSO_4$ and concentrated . Addition of pentane precipitated 3 (297 mg, 0.73 mmol, 78%) as an amber powder.
TLC Rf 0.1 (EtOAc); MS M+1=407, M-PO(OBn)$_2$=147; ^{1}H NMR δ 2.5 (d, 1H, H_1), 3.1 (s, 1H, OH), 3.3 (s, 1H, OH), 3.5 (s, 1H, OH), 3.6 (dd, 1H, H_4), 3.9 (m, 1H, H_5), 4.2 (m, 2H, H_6 & $H_{6'}$), 4.6 (t, 1H, H_3), 5 (m, 4H, CH_2Ar), 7.4 (s 10H, Ar); ^{13}C NMR δ 62.1 (C_3), 68.8 (C_2), 69.3 (C4), 69.3 (d, C_6, J=4,4 Hz), 69.8 (C_5), 72.1 & 73.6 (CH_2Bn), 81.8 (C_1), 127.4-128.1-134.7 (Ar). Anal calcd for $C_{20}H_{23}O_7P$: C 59.11, H 5.7, P 7.62 found C 58.62 H 5.85 P 7.73.

1,2-dideoxy-D-arabino-hex-1-enitol Dibenzyl 6-phosphate (4). Quinolein (50 µl) was added to a suspension of Lindlar catalyst (5% Pd on $CaCO_3$ poisonned with 3.5% lead) in a 100 ml round bottom flask containing ethyl acetate (10 ml). After degasing (10 mm Hg, 15 min) hydrogen was introduced in the flask (3 cycles). After stirring for 1 hour under an atmospheric pressure of hydrogen 3 (100 mg, 0.24 mmol) in ethyl acetate (5 ml) was added. After 3 additionnal purges, the mixture was stirred at room temperature for 30 min then filtered on celite. The organic solution was washed with 5% HCl (2x10 ml) which was back extracted with ethyl acetate (2x20 ml). The combined organic phases were washed to neutrality with water (40 ml) dried over $MgSO_4$ and evaporated under vacuum. The residue was redisolved in ethyl acetate (2 ml) and precipitated with pentane to give 4 (70 mg, 0.17 mmol, 70%) as a colorless solid contaminated with fully reduced triple bond (10%) (NMR).
TLC Rf 0.1 (EtOAc); HPLC (silica gel column 3.9 x 150 mm, flow rate 0.7 ml/min, 9:1 EtOAc/Heptane as mobile phase): t_{R4}= 4.2 min, t_R simple bond= 5.5 min; MS M+1=409, M-OBn=301; ^{1}H NMR δ 3.4 (dd, 1H, H_4), 3.8 (m, 1H, H_5), 4.2 (m, H_6 & $H_{6'}$), 4.4 (m , 1H, H_3), 5 (m, 4H, CH_2Bn) , 5,3 (m, 2H, H_1 & $H_{1'}$), 6.0 (m, 1H, H_2), 7.4 (s 10H, Ar); ^{13}C NMR δ 69.3-69.4 ($2CH_2$ Bn), 69.7-69.8 (d, C_6, J=6,6 Hz), 70.5-77.2 (m, C_2, C_3, C_4, C_5), 116.1 (C_1), 127.7-128.4 (m, Ar). Anal calcd for $C_{20}H_{25}O_7P$: C 58.82, H 6.17, found C 58.96 H6.24.

1,2-anhydro-D-glucitol Dibenzyl 6-phosphate (5). *m*CPBA (160 mg, 3.7 eq) was added to a solution of 4 (100 mg, 0.25 mmol) dissolved in CH_2Cl_2 (3 ml). After 4 hour stirring at room temperature, the solution was diluted to 10 ml and washed to neutrality with sat. aqueous $HNaCO_3$ and brine. After drying over MgSO4 and concentration the crude mixture was purified by flash chromatography (8:2 EtOAc/Heptane as eluent) and then by HPLC (C18, 65/35 Water/CH_3CN as mobile phase) to give 5 (70 mg, 0.17 mmol, 70%) as colorless cristals.
TLC Rf 0.4 (EtOAc); HPLC (C18 column 8x100 mm, flow rate 2 ml/min, water/acetonitrile 65/35 as mobile phase): t_{R5} = 7min, t_R simple bond = 5.5 min; MS (FAB, NaCl) M+1 = 425, M+Na= 448; ^{1}H NMR δ 2.8 (m, H_2), 2.9 (m, H_1), 3.2 (m, $H_{1'}$), 3.6 (m, H_4), 3.9 (m, H_3, H_5), 4.23 (dd, H_6 & $H_{6'}$), 5 .1 (m, 4H, CH_2Ar), 7.4 (s 10H, Ar); ^{13}C NMR δ 61.7-62.3 (C_6, diast.), 70.1-70.3 (CH_2 Bn), 77.5-77.7 (C_2, diast.), 79.4-80 (C_4, diast.), 82-82.4 (C_3, diast.), 83.8 (C_5), 128.2-128.9 (m, Ar). Anal calcd for $C_{20}H_{25}O_8P$: C 56.6, H 5.94, O 30.16, P 7.3, found C 56.71, H6.16, O 29.55, P 7.08.

1,2-anhydro-D-glucitol-6-phosphate (6). 5 (20 mg, 0.045 mmol) was added to a suspension of palladium 10% on charcoal (1.6 mg) in methanol (1 ml) containing triethylamine (5,7 mg, 1.25 eq.). After degasing under vacuum (3 times) the solution was stirred at room temperature for 40 min under H_2 (1 atm.). After filtration (celite) $HNaCO_3$ was added (0.122 ml of a 0.5 M solution, 1.3 eq.) and the solvent was removed under vacuum and the residue triturated in ether to give 6 as the disodium salt (15 mg, 70%).

MS (FAB, Thioglycerol, LiCl) M+1 = 289, M+2Li+1 = 303; ^1H NMR δ 2.75 (m, H_1 & $H_{1'}$), 3.2 (m, H_2), 3.6 (q, 1H, H_4) , 3.8 (m, 2H, H_3 & H_5), 4. (m, 2H, H_6 & $H_{6'}$,)

Titration of epoxide: 10.75 mg (0.373 mmol) of 6 was added to 0.208 ml of a neutralized 0.2 M sodium thiosulfate in 50% acetone containing phenolphthalein. The pink colour developed upon heating was discharged by adding 0.110 ml of 0.2 N acetic acid. 0.22 mmol (60%) of epoxide was titrated by this method.

1,2[^{3}H]-1,2-anhydro-D-glucitol-6phosphate.. The procedure described above was carried out under a tritium atmosphere. Compound 3 (10.2 mg, 0.025 mmol) was dissolved in ethyl acetate (1.5 ml) containing quinolein (5 µl). Tritiation (tritium gas: 0.37 TBq, 10 Ci) was conducted (via Toepler pump) over Linlar catalysis (5.8 mg) at room temperature during 25 min. Compound [^{3}H]-4 was obtained in 90% yield contaminated by 10% of [^{3}H]-4a and purified on silica gel HPLC (59 Ci/mmol).

HPLC (silica column, 4.6x250 mm, flow rate 1 ml/min, 70:30:2 AcOEt/Heptane/acetic acid as mobile phase): t_{R4}= 13,4 min, t_{R4a}= 17,3 min; MS (DCI/NH_3) M+1= 413 (59 Ci/mmol). Compound [^{3}H]-4 (50 mCi) was diluted 250 fold with 100 mg (0,25 mmol) of 4 to perform epoxidation as previously described and to obtain partly [^{3}H]-5 of SA= 15 mCi/mmol. After dilution with 5 (4 fold) hydrogenolysis was performed to obtain partly [^{3}H]-6 as described with no further purification, and used as such for enzyme labelling.

References

[1]. Ghosh S., Blumenthal H.J., Davidson E. & Roseman S.-J. Biol. Chem. 235: 1265-1273 (1960)
[2]. Gollinelli-Pimpaneau B., Le Goffic F. & Badet B.-J. Am. Chem. Soc. 111: 3029-3034 (1989)
[3]. Noltmann E.A.-The Enzymes 6: 271-354 (1972)
[4]. Landro J.A., Brush E.J. and Kozarich J.W.- Biochem. 31:6069-6077 (1992)
[5]. O'Connell E.L.& Rose I.A. - J. Biol. Chem. 248: 2225-2231 (1956)
[6]. Just G. & Potvin P. - Can. J. Chem 58: 2173-2177 (1980)
[7]. Colvin S., Hamill B.J.- J.C.S. Perkin I 869-874 (1977)
[8]. Lewbart M.L. & Schneider J.J.-J. Org. Chem. 34: 3505-3512 (1969)
[9]. Yu K.L. & Fraser-Reid B.-Tetrahedron Lett. 29: 979-982 (1988)
[10]. Ross W.C.J - J. Chem. Soc. 2257 (1950)

Acknowledgments

We are much indebted to Dr A. OLESKER for helpful discussions during the course of this work. Financial support from Ministère de la Recherche et de l'Enseignement Supérieur to C.L. is gratefully acknowledged.