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Note

Appel–Lee synthesis of glycosyl inositols, substrates for inositol dehydrogenase from *Bacillus subtilis*

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Abstract—We recently reported that inositol dehydrogenase (EC 1.1.1.18) from *Bacillus subtilis* can catalyze the highly stereoselective oxidation of 1L-4-O-substituted *myo*-inositol derivatives, as well as disaccharides melibiose and isomaltose, but not gentiobiose or maltose, consistent with the requirement of an α-(1→6) linkage. We believed that the enzyme might therefore catalyze efficient stereoselective oxidation of the appropriate α-linked glycosyl inositols. We have synthesized α-D-glucopyranosyl-(1→4)-(DL)-*myo*-inositol and α-D-galactopyranosyl-(1→4)-(DL)-*myo*-inositol using the Appel–Lee protocol to couple benzyl-protected glycosyl donors to protected inositols. This method failed in our hands using glycosyl donors derived from D-mannose and 2-azido-2-deoxy-D-glucose. When *myo*-inositol 1,3,5-monoorthoformate is used as the acceptor, the reaction is regiospecific for the 4/6-position. We report here the mildest conditions known for the removal of the orthoformate group. 2-Azido-2-deoxy-α-D-glucopyranosyl-(1→4)-(DL)-*myo*-inositol was synthesized using the trichloroacetimidate derivative as the donor, and all three pseudo-disaccharides were substrates for inositol dehydrogenase. The glucopyranosyl and galactopyranosyl derivatives displayed apparent second-order rate constants comparable to that of *myo*-inositol.

Keywords: Inositol dehydrogenase; Glycosyl inositol; Glycosylation

Glycosyl inositols are sugar-inositol conjugates found in prokaryotic and eukaryotic cells, both as small molecules and as components of complex structures. Examples of simple, naturally occurring glycosyl inositols include galactinol (α-D-galactopyranosyl-(1→1)-1D-*myo*-inositol), which acts as a galactosyl donor in the formation of glycosinolates;[1] mycothiol (1D-myo-inosityl 2-(*N*-acetylcysteinyl)amido-2-deoxy-α-D-glucopyranoside), a functional analog of glutathione found in mycobacteria and streptomycetes;[2] and a small group of D-galactosamine–inositol conjugates that are putative insulin mediators.[3] More complex examples are glycosylphosphatidylinositols (GPIs), which act as anchors for cell-surface proteins. GPIs typically consist of a glycan largely made up of mannose residues, but connected via a 2-amino-2-deoxy-D-glucose (GlcN) residue to the 1D-*myo*-inositol moiety of a phosphatidylinositol (PI) phospholipid.[4] The biosynthesis of GPIs has attracted attention as a target for anti-parasitic drugs, because protozoa, such as *Trypanosoma cruzei*, *Plasmodium falsiparum*, *Leishmania major*, and *Toxoplasma gondii*, synthesize these molecules via a different route from that found in mammalian cells.[4–6] The stereochemistry of the inositol moiety has been revealed as an exploitable feature in drug design: the GlcN-1L-PI has been demonstrated to inhibit GPI biosynthetic enzymes from *P. falsiparum* and *T. cruzei*.[7,8] Thus, the biological origin and fate of such molecules is of interest to the research community, and there is a continued need for the efficient regio- and stereoselective syntheses of glycosyl inositols.

We recently observed that *myo*-inositol dehydrogenase (IDH, EC 1.1.1.18) from *Bacillus subtilis* can not only catalyze the NAD^+-dependent oxidation of *myo*-inositol to *scyllo*-inosose, but it can also use as substrates, with high apparent stereoselectivity, the

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1L-4-O-substituted *myo*-inositol derivatives; the 1D-enantiomer being neither a substrate nor an inhibitor.[9] The substituent can be as large as a camphorsulfonyl group, and aromatic groups are recognized particularly well, resulting in apparent K_m values lower than that of *myo*-inositol! Because there could be no biological imperative for the oxidation of 1L-4-*O*-benzyl-*myo*-inositol, for example, we speculated that perhaps there was some naturally occurring compound that could take advantage of this cavity adjacent to the active site. Inositol-4-phosphate showed only trace activity, consistent with a relatively nonpolar cavity. Recalling that phenyl glycosides are known to bind more strongly to proteins than the disaccharides that are the in vivo ligands,[10,11] and knowing that IDH could also oxidize roughly isosteric monosaccharides, such as D-glucose, we proposed that disaccharides might also be oxidized by IDH. This proved to be the case: α-(1→6) linked disaccharides melibiose and isomaltose are substrates for the enzyme, while β-(1→6) linked gentiobiose and α-(1→4) linked maltose showed little or no activity. The kinetic constants obtained for these substrates were not so high as to suggest that IDH is truly a 'disaccharide dehydrogenase'. Recently it has been reported that D-*chiro*-inositol is also a substrate, but kinetic constants were not reported.[12]

From the results described above, it was clear that certain glycosyl inositols might be very efficient substrates for IDH. We predicted that compounds, such as D-glucopyranosyl-α-(1→4)-1L-*myo*-inositol (**1**) and D-galactopyranosyl-α-(1→4)-1L-*myo*-inositol (**2**), would act as substrates. The 1D-diastereomer will not react; therefore, the need for a stereospecific synthetic scheme is removed. Because several of the examples of biologically important glycosyl inositols noted above contain glucosaminyl-inositol, we were also interested in observing the action of IDH on 2-azido-2-deoxy-α-D-glucopyranosyl-(1→4)-*myo*-inositol (**3**), which serves as a synthetic precursor to glucosaminyl-inositol derivatives (Fig. 1).

The key consideration in the synthesis is the formation of the α-glycosidic bond. As discussed by Cid et al.,[13] the major requirement for the construction of the 1,2-*cis*-glycosidic bonds like the α-linkages we desired is the presence of a non-participating group at C-2 of the glycosyl donor. Thus, we chose the benzyl group to protect the C-2 oxygen of the glycosyl donor, except in the case of the 2-azido-2-deoxy-glucosyl donor. Martín-Lomas and co-workers have described the synthesis of many inosityl 2-azido-2-deoxy-α-D-glucopyranosides using the trichloroacetimidate (TCA) method.[13–15] Yields of such glycosylation reactions show 'fair to good stereoselectivity' and yields typically <50%. We hoped to improve on this by applying the Appel–Lee method of glycosidic bond formation[16,17] that has recently been exploited by Nishida and co-workers.[18–20] In general

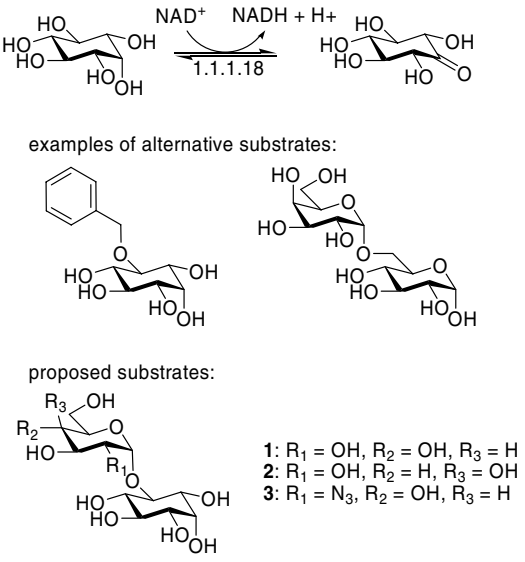
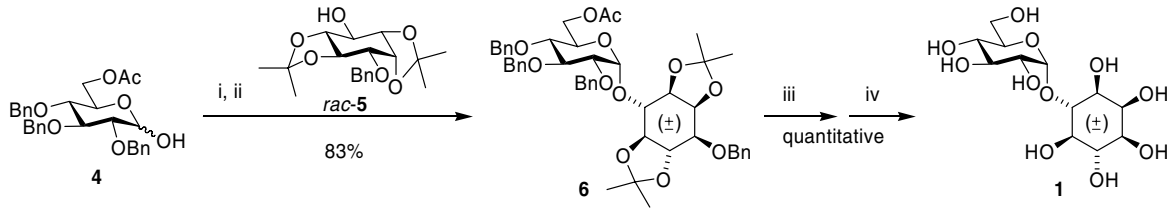


Figure 1.

terms, this method uses a tertiary phosphine and a carbon tetrahalide as a mild and efficient way of generating a glycosyl halide for a halide-catalyzed glycosylation.[21] The Appel–Lee coupling method, which we performed using triphenylphosphine and carbon tetrabromide, is far simpler to perform than using TCA derivatives. In particular, no precautions, such as an inert atmosphere and molecular sieves, are required to ensure dryness during the reaction. For the preparation of **1**, we synthesized a glucosyl donor 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose (**4**)[22] and a protected inositol (±)-3-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (**5**)[23] as the acceptor, using published methods. Using the Appel–Lee procedure, we isolated **6** as the expected pair of diastereomers in 83% yield, and contamination by the β-linked pseudo-disaccharide could not be detected by NMR spectroscopy. Deprotection by hydrogenolysis of the benzyl ethers, followed by methanolysis of the ester, proceeded in quantitative yields to give **1**. This represents a considerable improvement over the previously reported syntheses of glucosyl inositols.

It is well known that the alkylation and acylation of *myo*-inositol 1,3,5-monoorthoformate (**7**) can be achieved with high regioselectivity, owing to the pK_a difference of the axial 4/6-hydroxyl group relative to the hydroxyl group of carbon-2.[9,24] We hoped that this glycosylation reaction would result in a similar regioselectivity, because **7** is commercially available, and very easy to prepare from *myo*-inositol. Using 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose **8**[25] as the glycosyl donor, reaction with **7** provided **9a** as an 82:18 mixture of α:β anomers, as discerned by ^{1}H NMR, in 81% yield (Scheme 2). This matches the findings of anomeric selectivity for the galactopyranosyl reaction reported previously.[18] The reaction proceeded to completion much



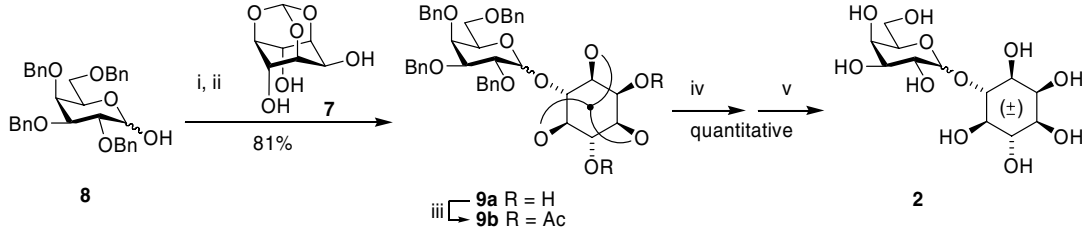
Scheme 1. Synthesis of **1**. Reagents and conditions: (i) PPh_3, CBr_4, CH_2Cl_2, rt, 3 h; (ii) tetramethylurea, *rac*-**5**, rt, 1 week; (iii) 1 atm H_2, 10% Pd/C, CH_3OH, rt, 2 d; (iv) CH_3ONa/CH_3OH, rt, 2 h.

more quickly than that of Scheme 1, due at least in part to the more polar solvent system,[20] but perhaps also due to the enhanced reactivity of **7**. To deconvolute the NMR signals and establish the site of glycosylation, the product was per-acetylated to **9b**, allowing us to distinguish the glycosylation of the 2-position of inositol from the glycosylation of the 4-position. The former would result in a *meso*-product, with H-4/6 giving one signal in the proton spectrum. The isolated product **9b** is unsymmetrical, resulting in two signals at 5.5 and 5.4 ppm, confirming the expected regioselectivity. Hydrogenolysis of **9a** removed the benzyl groups, as discussed previously. The orthoformate ester was cleaved by stirring in water in the presence of Dowex 50X8 (H^+-form) at room temperature overnight, resulting in **2** as a mixture of anomers. This procedure is milder and simpler than the usual method using 80% trifluoroacetic acid.

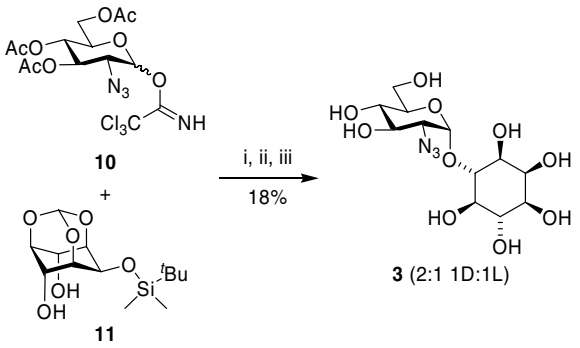
Attempts to use the Appel–Lee procedure for the coupling of suitably protected mannose derivatives to **7** were unsuccessful using 2,3,4,6-tetra-*O*-acetyl-D-mannopyranose or 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-mannopyranose. NMR and TLC analysis suggested the mannopyranosyl bromide formed, which did not react further under these conditions. Using 2-azido-2-deoxy-3,4,6-tri-*O*-benzyl-D-glucopyranose as the donor, no product was isolated. In this case, triphenylphosphine may react with the azido group to form an iminophosphorane. We therefore synthesized **3** by the reaction of **10** with **11**. Donor **10** was synthesized from 2-amino-2-deoxy-D-glucose by diazo transfer, followed by protection and activation as the TCA derivative.[26,27] Acceptor **11**[28] bears a protecting group at C-2 because this glycosylation is not regioselective.[29] The coupling and deprotection proceeds in a modest yield, resulting in a 2:1 mixture of 4-*O*- and 6-*O*-glycosyl diastereomers (Scheme 3).

As we predicted, **1** acted as a substrate for IDH under the conditions we have described previously.[9] The apparent V_{max} and K_m values were 4.0 ± 0.5 μmol min^{-1} mg^{-1} and 18 ± 2 mM, respectively, comparable to those observed for *myo*-inositol (8 ± 1 μmol min^{-1} mg^{-1} and 18 ± 1 mM). We predicted that the 1D-inositol isomer of **1** would act as neither a substrate nor an inhibitor, in simile with the behaviour of 4-*O*-benzyl-*myo*-inositol. The ^1H NMR spectrum of an enzymatic reaction in which NADH is recycled in the presence of lactate dehydrogenase and excess pyruvate[9] confirmed that only one diastereomer reacts. Consistent with our understanding of the IDH active site as described above, this suggests that the true K_m value for the 1L-isomer of **1** is 9 mM, and therefore the apparent second-order rate constants (V/K) for the oxidation of *myo*-inositol and 1L-**1** are the same. Because **2** and **3** are not simple mixtures of 1D- and 1L-stereoisomers, the uncertainty associated with any measured kinetic constants becomes significant. If our assumption that neither the 1D-isomers nor the β-anomers serve as substrates, then **2** shows similar reactivity to that of **1**, and each of these is five times more reactive than **3**. The steric demand of the azido-group, the only significant difference between the substrates, must impair the fit in the active site.

In conclusion, the Appel–Lee procedure can be applied efficiently to the synthesis of glucopyranosyl and galactopyranosyl inositols, and we hope this advance will allow improved synthesis of more complex targets. We have shown that IDH can catalyze the oxidation of glycosyl inositols efficiently; it may be that IDH acts as a broad-spectrum inositol/pyranose dehydrogenase



Scheme 2. Synthesis of **2**. Reagents and conditions: (i) PPh_3, CBr_4, DMF/CH_2Cl_2 (1/1), rt, 3 h; (ii) tetramethylurea, **7**, rt, 20 h; (iii) Ac_2O/pyridine (1/1), rt, 16 h; (iv) 10% Pd/C, CH_3OH, 1 atm H_2, rt, 2 days; (v) Dowex 50X8 (H^+-form), H_2O, rt, 2 h.



Scheme 3. Synthesis of **3**. Reagents and conditions: (i) TMSOTf (0.1 equiv), CH_2Cl_2, rt, 1 h; (ii) CH_3ONa/CH_3OH, rt, 2 h; (iii) Dowex 50X8 (H^+-form), H_2O, 16 h.

to allow the organism to take advantage of the available carbon sources.

1. Experimental

1.1. General methods

Chemical reagents, including buffers, salts, *myo*-inositol, D-glucose, D-galactose, and NAD^+ were obtained from Sigma–Aldrich Canada, Ltd (Oakville, ON), or VWR CanLab (Mississauga, ON), and were categorized as Molecular Biology Grade or were the highest grade available. Inositol dehydrogenase was purified as we described elsewhere.[9] UV–vis absorbance was measured using a Beckman DU-640 spectrophotometer with a circulating water bath-controlled temperature block. NMR spectra were recorded on a Bruker 500 MHz spectrometer. Chemical shifts are reported in ppm downfield from tetramethylsilane. For the NMR spectra of the glycosyl inositols, signals from the pyranosyl moiety are labeled with prime (e.g., H-1′) and, when possible, signals arising from the 1D- and 1L-inositol stereoisomers are labeled a and b, respectively. TOCSY experiments were used to make assignments where necessary. Mass spectra were recorded with a VG 70SE mass spectrometer. Thin-layer chromatography was performed on aluminum-backed plates of Silica Gel $60F_{254}$ (EM Science, Gibbstown, NJ) using phosphomolybdic acid/ethanol reagent, or a 10% solution of sulfuric acid in ethanol, and/or UV at 254 nm to visualize the spots. Silica Gel 60 (40–63 μm) was used for flash chromatography.

1.2. General protocol for one-pot glycosylation using the Appel–Lee reagents

Reactions were carried out in a glass vessel closed with a septum. Neither molecular sieves nor an inert atmosphere was used. A 2-*O*-benzyl-1-hydroxy sugar in CH_2Cl_2 (for **1**) or in a 1/1 mix of CH_2Cl_2/DMF (for **2**) was treated with Ph_3P (3 mol equiv) and CBr_4 (3 mol equiv) and stirred for 3 h at rt. Then, *N*,*N*-tetramethylurea and the acceptor alcohol (3 mol equiv) were added and stirred at rt. In both cases, the reaction was continued until the bromide donor was consumed completely, as evidenced by TLC analysis. The reaction mixture, diluted with $CHCl_3$, was washed with satd aq $NaHCO_3$ and aq NaCl solution, dried over anhydrous $MgSO_4$, and concentrated. The product was purified by silica gel column chromatography.

1.3. 6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-(1→6)-(±)-1,2,4,5-di-*O*-isopropylidene-3-*O*-benzyl-*myo*-inositol (6)

This compound has been prepared from **4** (0.60 g, 1.2 mmol) and **5** (1.28 g, 3.63 mmol) following the Appel–Lee protocol to yield 0.82 g of **6** (83%), as a 1/1 mixture of diastereoisomers. ^1H NMR (500 MHz, $CDCl_3$): δ 7.45–7.25 (m, 5H × 8, $-CH_2C_6H_5$), 5.46 (d, 1H, $J = 4.0$ Hz, H-1′a), 5.41 (d, 1H, $J = 4.0$ Hz, H-1′b), 5.05 (dd, 2H, $J = 2.0$, 10.0 Hz), 4.94–4.80 (m, 11H), 4,73–4.53 (m, 7H), 4.48–4.31 (m, 5H), 4.25–3.90 (m, 16H), 3.80–3.75 (m, 2H), 3.60–3.45 (m, 6H), 3.35 (t, 1H, $J = 10$ Hz), 2.03, 2.02 (2s, 3H × 2, CH_3 acetate), 1.58, 1.56, 1.52, 1.49, 1.46, 1.43, 1.39, 1.32 (8s, 3H × 8, CH_3 isopropylidene); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.18, 171.15 (2 × C=O), 139.0–138.0, 128.9–128.0, 112.79 (O–*C*–O), 112.52 (O–*C*–O), 110.46 (O–*C*–O), 110.32 (O–*C*–O), 96.58 (C-1′a), 95.73 (C-1′b), 82.36, 82.12, 81.93, 80.08, 79.69, 79.57, 79.41, 79.18, 78.02, 77.78, 76.86, 76.15, 76.09, 75.57, 75.53, 75.33, 74.86, 74.80, 72.81, 72.45, 72.37, 68.58, 68.50, 63.29, 63.16, 60.77, 28.72, 28.61, 27.54, 27.51, 27.39, 27.32, 26.42, 26.22 (8 × *C*H_3 isopropylidene), 21.29, 21.23 (2 × *C*H_3 acetate); ESIMS: *m*/*z* calcd for $C_{48}H_{56}O_{12}Na$ $[M+Na]^+$: 847.3669. Found: 847.3661.

1.4. α-D-Glucopyranosyl-(1→6)-(±)-*myo*-inositol (1)

Compound **6** (0.82 g, 1 mmol) and Pd/C 10% (200 mg) in CH_3OH (50 mL) were stirred for 2 days under an atmosphere of H_2. The reaction mixture was filtered through Celite, and a catalytic amount of $NaOCH_3$ was added. The solution was stirred for 2 h at rt, then neutralized using Dowex 50X8 (H^+-form) and concentrated. The residue was re-taken in water and lyophilized to yield 340 mg of **1** (99%), as a 1/1 mixture of diastereoisomers. All NMR spectra were in good agreement with those previously reported.[30,31] ^1H NMR (500 MHz, D_2O): δ 5.22 (d, 1H, $J = 4.0$ Hz, H-1′a), 5.18 (d, 1H, $J = 4.0$ Hz, H-1′b), 3.96 (t, 1H, $J = 2.7$ Hz, H-2a), 3.94 (t, 1H, $J = 2.7$ Hz, H-2b), 3.93–3.88 (m, 2H), 3.70–3.20 (m, 20H); ^{13}C NMR (125 MHz, D_2O): δ 99.84 (C-1′a), 99.81 (C-1′b), 81.00

(2 × C-4), 75.00 (C-5a), 73.39 (C-3′a), 73.34 (C-3′b), 73.29 (C-5b), 72.94 (C-2a), 72.86 (C-6b), 72.80 (C-2b), 72.63 (C-5′a), 72.61 (C-5′b), 72.32 (C-6a), 72.23 (C-2′a), 72.13 (C-2′b), 72.00 (C-3b), 71.37 (C-1a), 71.35 (C-1b), 70.15 (C-3a), 69.81 (C-4′a), 69.70 (C-4′b), 60.80 (C-6′a), 60.66 (C-6′b); ESIMS (ESI): *m*/*z* calcd for $C_{12}H_{21}O_{11}$ $[M-H]^-$: 341.1089. Found: 341.1085. Anal. Calcd for $C_{12}H_{22}O_{11}+H_2O$: C, 40.00; H, 6.71. Found: C, 40.16; H, 6.65.

1.5. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→4)-(±)-1,3,5-*O*-methylidyne-*myo*-inositol (9a)

This compound was prepared from **8** (1.5 g, 2.8 mmol) and **7** (1.6 g, 7.8 mmol) following the Appel–Lee protocol to yield 1.6 g of **9a** (81%), as a 82/18 α/β mixture of anomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.25 (m, 5H × 4, $-CH_2C_6H_5$), 5.54 (d, 1H, $J = 1.2$ Hz, H orthoformate anomer β), 5.52 (d, 1H, $J = 1.2$ Hz, H orthoformate anomer α), 5.14 (d, 1H, $J = 3.0$ Hz, H-1α′), 5.00–4.96 (m, 1H), 4.90–4.83 (m, 2H), 4.80–4.66 (m, 3H), 4.60–4.56 (m, 2H), 4.51–4.41 (m, 4H), 4.38–4.32 (m, 2H), 4.27 (t, 1H, $J = 6.0$ Hz, H-5′), 4.18–4.00 (m, 4H), 3.67–3.42 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) of the α-anomer: δ 139.27, 139.08, 138.94, 138.45, 129.00–127.80, 102.88 (C orthoformate), 98.91 (C-1′), 79.34, 76.99, 75.62, 75.24, 73.76, 73.59, 73.55, 73.37, 72.79, 70.15, 69.91, 69.09, 68.48, 68.44, 68.00; ESIMS *m*/*z* calcd for $C_{41}H_{44}O_{11}Na$ $[M+Na]^+$: 735.2775. Found: 735.2780.

1.6. 2,3,4,6-Tetra-*O*-benzyl-D-galactopyranosyl-(1→4)-(±)-2,6-di-*O*-acetyl-1,3,5-*O*-methylidyne-*myo*-inositol (9b)

An aliquot of **9a** was quantitatively per-acetylated by the overnight action of a 1/1 (v/v) mixture of Ac_2O/pyridine. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.25 (m, 5H × 4, $-CH_2C_6H_5$), 5.58 (d, 1H, $J = 1.2$ Hz, H orthoformate anomer α), 5.49 (m, 1H, H-6), 5.38 (m, 1H, H-2), 5.02 (d, 1H, $J = 3.0$ Hz, H-1α′), 5.00–4.96 (m, 1H), 4.90–4.83 (m, 2H), 4.80–4.66 (m, 3H), 4.60–4.41 (m, 6H), 4.35–4.32 (m, 1H), 4.24 (t, 1H, $J = 6.0$ Hz, H-5′), 4.12–4.08 (m, 2H), 3.90–3.85 (m, 1H), 3.58–3.46 (m, 2H), 2.07 (s, 3H, CH_3 acetate), 1.98 (s, 3H, CH_3 acetate); ESIMS: *m*/*z* calcd for $C_{45}H_{48}O_{13}Na$ $[M+Na]^+$: 819.2987. Found: 819.3000.

1.7. D-Galactopyranosyl-(1→6)-(±)-*myo*-inositol (2)

Compound **9a** (0.42 g, 0.59 mmol) and Pd/C 10% (200 mg) in CH_3OH (50 mL) were stirred for 2 days under an atmosphere of H_2. The reaction mixture was filtered through Celite, and concentrated. The residue was dissolved in water (50 mL) and 2 mL of Dowex 50X8 (H^+-form) was added. After stirring at rt overnight and filtration, the solution was lyophilized to yield 201 mg of **2** (99%), as a mixture of anomers α/β 82/18. ^{1}H NMR (500 MHz, D_2O): δ 5.09 (d, 1H, $J = 3.4$ Hz, H-1′α), 4.47 (d, 1H, $J = 7.6$ Hz, H-1′β), 4.10 (t, 1H, $J = 6.0$ Hz, H-5′), 4.03 (t, 1H, $J = 2.0$ Hz), 3.95 (t, 1H, $J = 2.7$ Hz, H-2), 3.90 (m, 1H), 3.77 (m, 2H), 3.65–3.45 (m, 4H), 3.42 (dd, $J = 3.0$ Hz, $J = 9.0$ Hz, 1H), 3.17 (m, 1H); ^{13}C NMR (125 MHz, D_2O) of the α anomer: δ 103.56 (C-1′), 84.49 (C-4), 76.98, 75.59, 75.35, 74.78, 74.39, 73.94, 73.23, 72.06, 71.75, 71.63, 63.52 (C-6′); ESIMS *m*/*z* calcd for $C_{12}H_{22}O_{11}Na$ $[M+Na]^+$: 365.1054. Found: 365.1064.

1.8. 2-Azido-2-deoxy-α-D-glucopyranosyl-(1→6)-(±)-*myo*-inositol (3)

Toluene was evaporated three times from a mixture of **10** (100 mg, 0.21 mmol) and **11** (50 mg, 0.16 mmol), and the residue was dried under vacuum for several hours. Freshly distilled CH_2Cl_2 (2.5 mL) was added and the mixture was stirred at rt for 30 min. Then, a solution of TMSOTf in CH_2Cl_2 (0.2 M, 0.1 mL, 0.1 equiv) was added and the solution was stirred at rt for 1 h. The reaction was quenched by the addition of Et_3N (0.3 mL), and concentrated. Flash chromatography (hexane/EtOAc, 1/1) of the residue gave a partially desilylated mixture of the pseudo-disaccharide. This was deacetylated using a catalytic amount of $NaOCH_3$ in CH_3OH (5 mL) for 2 h at rt. Then, 2 mL of Dowex 50X8 (H^+-form) was added to neutralize to $NaOCH_3$, and also to remove the other protecting groups. After stirring at rt overnight and filtration, the solution was concentrated. The residue was extracted in water and lyophilized to yield 11.1 mg of **12** (18%), as a 2/1 mixture of diastereoisomers. ^{1}H NMR (500 MHz, D_2O): δ 5.38 (d, 1H, $J = 3.6$ Hz, H-1′a), 5.32 (d, 1H, $J = 3.6$ Hz, H-1′b), 3.99–3.95 (m, 2H), 3.94 (t, 1H, $J = 2.7$ Hz, H-2a), 3.91 (t, 1H, $J = 2.7$ Hz, H-2b), 3.93–3.88 (m, 2H), 3.70–3.20 (m, 16H); ESIMS: *m*/*z* calcd for $C_{12}H_{20}O_{10}N_3Na$ $[M+Na]^+$: 390.1119. Found: 390.1130.

1.9. Enzymatic studies

IDH was purified and kinetic assays were performed as previously described.[9] The enzymatic reaction as followed by NMR spectroscopy was prepared as follows: NAD^+ (0.6 mg, 1.5 mM), pyruvate (6.3 mg, 95 mM), and **1** (5.3 mg, 26 mM) were dissolved in 580 μL of 0.1 M Tris–DCl in D_2O, pD = 9.0, and the mixture was introduced into an NMR tube. Then, 1 U of lactate dehydrogenase (LDH) and 10 μL of IDH (8 mg/mL) were introduced. After 24 h, an additional 1 U of LDH as well as 10 mg of pyruvate and 10 μL of IDH dissolved in 50 μL of buffer were added to drive the reaction toward completion.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.05.001.

References

1. Noguchi, K.; Okuyama, K.; Ohno, S.; Hidano, T.; Wakiuchi, N.; Tarui, T.; Tamaki, H.; Kishihara, S.; Fujii, S. *Carbohydr. Res.* **2000**, *328*, 241–248.
2. Newton, G. L.; Fahey, R. C. *Arch. Microbiol.* **2002**, *178*, 388–394.
3. Brautigan, D. L.; Brown, M.; Grondrod, S.; Chinigo, S.; Kruszewski, A.; Lukasik, S. M.; Busheller, J. H.; Horal, M.; Keller, S.; Tamura, S.; Heimark, D. B.; Price, J.; Larner, A. N.; Larner, J. *Biochemistry* **2005**, *44*, 11067–11073.
4. Kinoshita, T.; Inoue, N. *Curr. Opin. Chem. Biol.* **2000**, *4*, 632–638.
5. Ferguson, M. A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 16479–16486.
6. Santos de Macedo, C.; Shams-Eldin, H.; Smith, T. K.; Schwartz, R. T.; Azzouz, N. *Biochimie* **2003**, *85*, 465–472.
7. Smith, T. K.; Cottaz, S.; Brimacombe, M. A.; Ferguson, M. A. *J. Biol. Chem.* **1996**, *271*, 6476–6482.
8. Smith, T. K.; Paterson, M. J.; Crossman, A.; Brimacombe, M. A.; Ferguson, M. A. *Biochemistry* **2000**, *39*, 11801–11807.
9. Daniellou, R.; Phenix, C. P.; Tam, P. H.; Laliberte, M. C.; Palmer, D. R. J. *Org. Biomol. Chem.* **2005**, *3*, 401–403.
10. Sahin-Toth, M.; Gunawan, P.; Lawrence, M. C.; Toyokuni, T.; Kaback, H. R. *Biochemistry* **2002**, *41*, 13039–13045.
11. Sujatha, M. S.; Sasidhar, Y. U.; Balaji, P. V. *Biochemistry* **2005**, *41*, 8554–8562.
12. Yoshia, K.; Yamaguchi, M.; Morinaga, T.; Ikeuchi, M.; Kinehara, M.; Ashida, H. *Appl. Environ. Microbiol.* **2006**, *72*, 1310–1315.
13. Cid, M. B.; Alfono, F.; Martin-Lomas, M. *Chem. Eur. J.* **2005**, *11*, 928–938.
14. Dietrich, H.; Espinosa, J. F.; Chiara, J. L.; Jiminéz-Barbaro, J.; León, Y.; Varela-Nieto, I.; Mato, J. M.; Cano, F. H.; Foces-Foces, C.; Martín-Lomas, M. *Chem. Eur. J.* **1999**, *5*, 320–335.
15. Martín-Lomas, M.; Khiar, N.; Garcia, S.; Koessler, J. L.; Nieto, P. M.; Rademacher, T. W. *Chem. Eur. J.* **2000**, *6*, 3608–3621.
16. Appel, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 801–811.
17. Lee, J. B.; Nolan, T. J. *Can. J. Chem.* **1966**, *44*, 1331–1334.
18. Nishida, Y.; Shingu, Y.; Dohi, H.; Kobayashi, K. *Org. Lett.* **2003**, *5*, 2377–2380.
19. Shingu, Y.; Nishida, Y.; Dohi, H.; Kobayashi, K. *Org. Biomol. Chem.* **2003**, *1*, 2518–2521.
20. Shingu, Y.; Miyachi, A.; Miura, Y.; Kobayashi, K.; Nishida, Y. *Carbohydr. Res.* **2005**, *340*, 2236–2244.
21. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
22. Fuerstner, A.; Albert, M.; Mlynarski, J.; Matheu, M. *J. Am. Chem. Soc.* **2002**, *124*, 1168–1169.
23. Chung, S.-K.; Ryu, Y. *Carbohydr. Res.* **1994**, *258*, 145–167.
24. Lee, H. W.; Kishi, Y. *J. Org. Chem.* **1985**, *50*, 4402–4404.
25. Arya, P.; Barkley, A.; Randell, K. D. *J. Comb. Chem.* **2002**, *4*, 193–198.
26. Alper, P. B.; Hung, S. C.; Wong, C. H. *Tetrahedron Lett.* **1996**, *37*, 6029–6032.
27. Rele, S. M.; Iyer, S. S.; Baskaran, S.; Chaikof, E. L. *J. Org. Chem.* **2004**, *69*, 9159–9170.
28. Angyal, S. J. *Carbohydr. Res.* **2000**, *325*, 313–320.
29. Uhlmann, P.; Vasella, A. *Helv. Chim. Acta* **1992**, *75*, 1979–1994.
30. Watanabe, Y.; Nakamoto, C.; Ozaki, S.; Sato, M.; Koizumi, K. *J. Carbohydr. Chem.* **1993**, *12*, 685–692.
31. Sato, M.; Nakamura, K.; Yagi, Y.; Koizumi, K. *Biotechnol. Lett.* **1992**, *14*, 659–664.