BIOCHEMICAL STUDIES ON 2-DEOXY-*SCYLLO*-INOSOSE, AN EARLY INTERMEDIATE IN THE BIOSYNTHESIS OF 2-DEOXYSTREPTAMINE

I. CHEMICAL SYNTHESIS OF 2-DEOXY-*SCYLLO*-INOSOSE AND [2,2-$^2H_2$]-2-DEOXY-*SCYLLO*-INOSOSE

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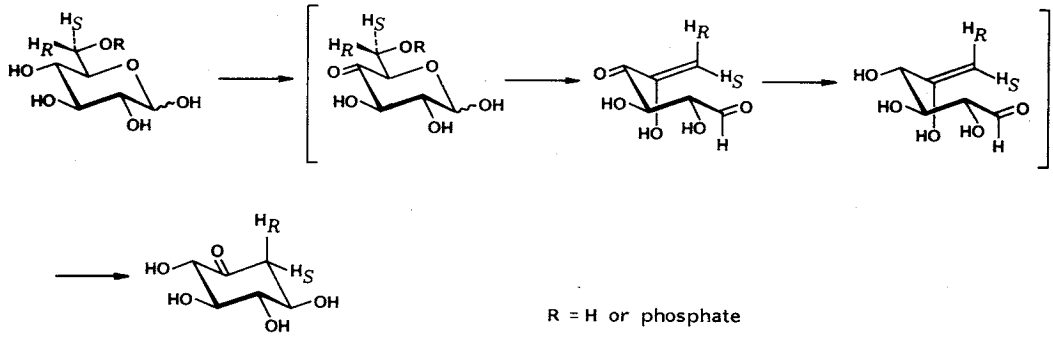
A practical preparative method for 2-deoxy-*scyllo*-inosose, the earliest key intermediate leading to 2-deoxystreptamine, was devised as a prerequisite to more detailed biochemical studies on the biosynthesis of 2-deoxystreptamine. [2,2-$^2H_2$]-2-Deoxy-*scyllo*-inosose was also synthesized through a modified Ferrier reaction.

2-Deoxystreptamine is a quite specific and important cyclitol in that it is involved as the central aglycon in the major group of clinically useful aminoglycoside antibiotics.[1] Further, 2-deoxystreptamine has been found only in these antibiotics and is supposed to be a typical microbial secondary metabolite directly biosynthesized from D-glucose. The earliest and crucial transformation in the biosynthesis of 2-deoxystreptamine is an intramolecular C-C bond formation between the C-1 and the C-6 carbons of D-glucose.[2,3] The mechanism and stereochemistry of this C-C bond formation is well established,[4~7] *i.e.* an aldol-type stereospecific cyclization affords 2-deoxy-*scyllo*-inosose (**1**) as the first six-membered carbocyclic product.

In view of the six-membered carbocyclic ring construction in the living systems, this 2-deoxy-*scyllo*-inosose formation reaction (secondary metabolism) is mechanistically similar to the dehydroquinate synthase reaction in the shikimate pathway (primary metabolism).[4~10] Comparative studies of primary and secondary metabolism from the chemical as well as biochemical viewpoints are quite intriguing and important to get further insight into secondary metabolisms. This may well lead to development of new applications in the production of materials. In this context, we became interested in biochemical studies on the 2-deoxy-*scyllo*-inosose-forming reaction in the microorganisms which are capable of producing 2-deoxystreptamine-containing antibiotics. Our first aim was to establish a cell-free reaction system for this transformation.

In order to construct an *in vitro* system for any biochemical reaction it is essential to establish a convenient assay system which allows one to trace the targeted reaction qualitatively and quantitatively. Generally, any biochemical reaction can be monitored either by measuring the changes of substrate(s), product(s), cofactor(s), and/or by assaying the biological activities, if any. The latter possibility cannot be applied for the biochemical formation of 2-deoxy-*scyllo*-inosose because 2-deoxy-*scyllo*-inosose is not known so far to have any biological activity including antimicrobial activity. In relation to the alternate approach, according to the proposed reaction mechanism of 2-deoxy-*scyllo*-inosose formation shown in Fig. 1, the substrate involved is presumably D-glucose-6-phosphate, while a cofactor may be nicotinamide

Fig. 1. The postulated mechanism of 2-deoxy-*scyllo*-inosose formation in the biosynthesis of 2-deoxystreptamine.



adenine dinucleotide (NAD) or its phosphate (NADP).[4,5] However, since D-glucose and D-glucose-6-phosphate are substrates for various biochemical reactions in primary metabolism tracing either of them cannot be suited for monitoring this particular 2-deoxy-*scyllo*-inosose-forming reaction unless a pure enzyme is used. The same is true for both of the nicotinamide cofactors as well. Therefore, the most suitable and reliable method to assay the reaction can be one based on the direct detection of the product, 2-deoxy-*scyllo*-inosose. One suitable analytical method seems to be a GC-MS selected ion monitoring (SIM) technique. In order to develop a convenient analytical method, a sufficient amount of 2-deoxy-*scyllo*-inosose itself is required in order to assess applicability of its derivatives for the GC-MS analysis, to determine the mass spectral fragmentation patterns, and to use as a standard in quantitative analysis.

Preparation of 2-deoxy-*scyllo*-inosose has been reported so far by two groups. DAUM *et al.* used the microbial oxidation methodology of viboquercitol with *Acetobactor suboxydanse*.[11] HORI *et al.* prepared radioactively-labeled 2-deoxy-*scyllo*-inosose by oxidation of 2-deoxy-*scyllo*-inosamine, which was accumulated by a mutant of a sagamicin producing *Micromonospora sagamiensis*.[12] However, neither way of acquiring 2-deoxy-*scyllo*-inosose is feasible, since viboquercitol is quite costly and the microbial oxidation technology is tedious, and 2-deoxy-*scyllo*-inosamine is almost impossible to acquire due to the requirement of special idiotrophs for production. As a consequence, we needed to develop a convenient chemical method of 2-deoxy-*scyllo*-inosose synthesis.

Furthermore, in order to use it as an internal standard for quantification and for determining the mass spectral fragmentations of 2-deoxy-*scyllo*-inosose derivatives, isotopically labeled derivatives are required. Suitable derivatives must have significant mass differences from the non-labeled specimen. We chose [2,2-$^2H_2$]-2-deoxy-*scyllo*-inosose as the standard. The synthesis of the labeled inosose was thus carried out by making use of the Ferrier reaction as a key step.

Results and Discussion

Synthesis of 2-Deoxy-*scyllo*-inosose

Myo-inositol was selected as a starting material for synthesis of 2-deoxy-*scyllo*-inosose, because not only it is commercially available and inexpensive, but selective protection of its hydroxyl groups can be performed depending upon the reactivity differences of axial and equatorial orientations.

Preparation of 2-deoxy-*scyllo*-inosose from *myo*-inositol seemingly involves a kind of intramolecular disproportionation, that is, the oxidation of an alcoholic function into a ketone and the reduction of another hydroxyl group to a methylene group. All the remaining four hydroxyl groups must be controlled to be equatorially oriented. Our preparation method is summarized in Scheme 1. *Myo*-inositol (**2**) was first transiently protected selectively by the GIGG's method to give 1,2-*O*-isopropylidene-*myo*-inositol (**3**),[13] since the C-1 and C-2 hydroxyl groups seemed to be an appropriate target site for the above-mentioned manipulation. The resulting tetraol **3** was then fully protected as a tetra-*O*-benzyl (Bn) ether **4**. The 1,2-*O*-isopropylidene group of **4** was then removed by acid hydrolysis. The crucial transformation was a conversion of the diol **5** into an olefin. Although several methods were available for conversion of a diol to an olefin, we used the TIPSON-COHEN's method,[14] because of its simplicity. Thus, the diol **5** was converted to a di-*O*-mesylate **6**, which was subsequently treated with Zn/NaI in refluxing *N*,*N*-dimethylformamide (DMF) to give an olefin **7**. This C_2 symmetric olefin **7** was previously prepared in an optically active form by OZAKI *et al.* from D-glucuronolactone in 16 steps.[15]

Functionalization of **7** was the next problem. Originally, oxymercuration to **7** was attempted as a single step conversion, but no reaction took place and the starting material **7** was recovered. A two-steps conversion was alternatively carried out. Treatment of **7** with *m*-chloroperoxybenzoic acid (*m*-CPBA) encountered a slight difficulty. The reaction proceeded rather slowly and use of a large excess of reagent caused side reactions. The optimum yield was obtained by using 1.5~2 equivalents of the oxidizing reagent and by quenching the reaction when about a half of the starting material was consumed. In this manner, the epoxide **8** was obtained in 44% yield and 42% of **7** was recovered. This epoxide **8** was reduced with $LiAlH_4$ to give an alcohol **9** as a single isomer. The orientation of this newly formed hydroxyl group at **9** was presumed to be axial, based on the general tendency of the direction of hydride attack on an epoxide in the six-membered ring, but was not rigorously assigned. This hydroxyl group was then oxidized with pyridinium chlorochromate in methylene chloride to give a ketone **10**, *i.e.* the fully benzylated 2-deoxy-*scyllo*-inosose. The final deprotection was carried out by catalytic hydrogenation over palladium on charcoal in a methanol - acetic acid - water solvent to yield the titled compound **1**. In ^2H_2O solution, the compound was shown by the ^{13}C NMR spectrum to exist in a keto-hydrate equilibrium, the ratio of which was estimated to be *ca.* 3 : 2. Since this compound was relatively unstable, the structure was characterized and verified as its tetra-*O*-trimethylsilyl (TMS) ether derivative **11**.

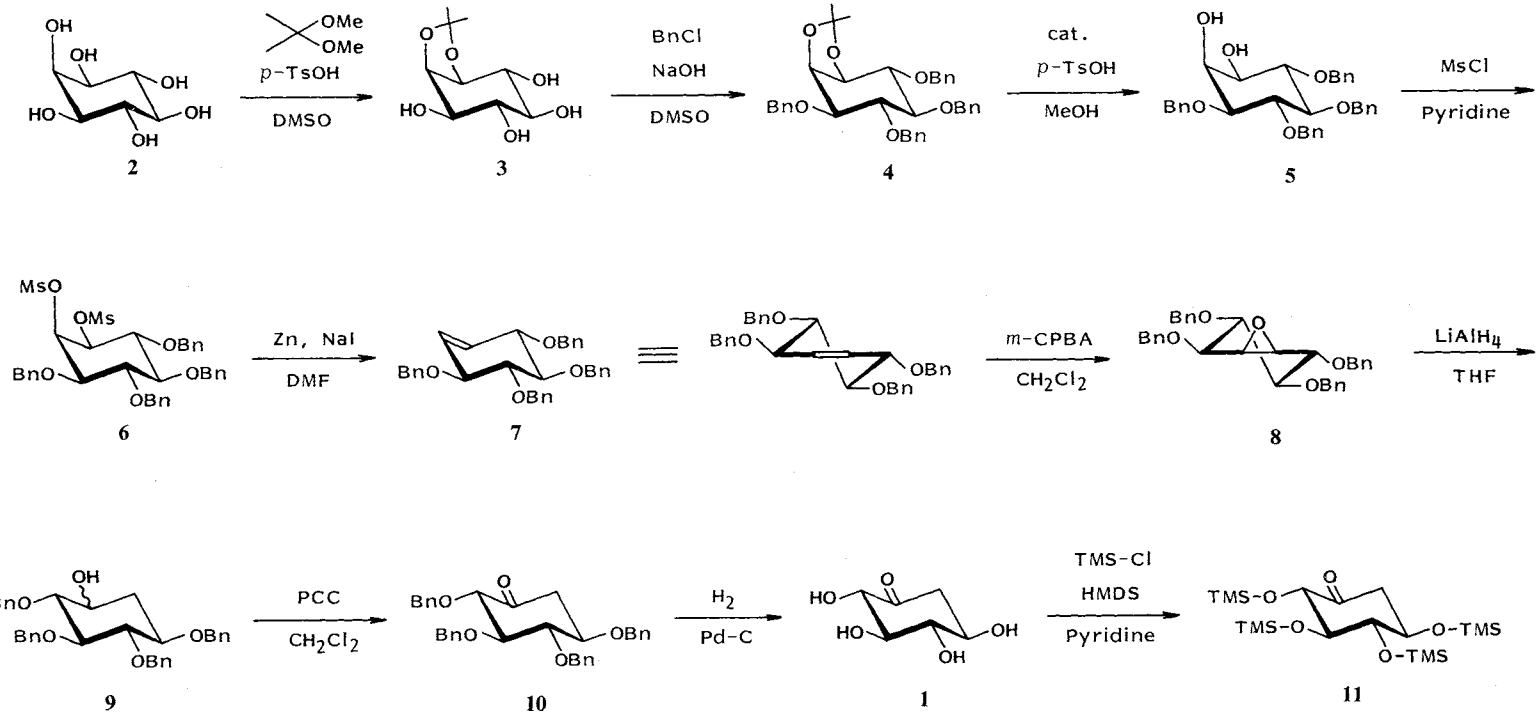
The overall yield was 9.6% in a total of 9 steps, which involve rather easy operations. It seems appropriate to note a synthetic potential of the present method. During the past several years, a series of *myo*-inositol phosphates and inhibitors of their metabolism have attracted wide attention due to their biological significance as the second messenger of biological signal transduction.[16,17] Some of those intriguing compounds may be prepared with minor modifications from the intermediates described in the present study.

Synthesis of [2,2-$^2H_2$]-2-Deoxy-*scyllo*-inosose

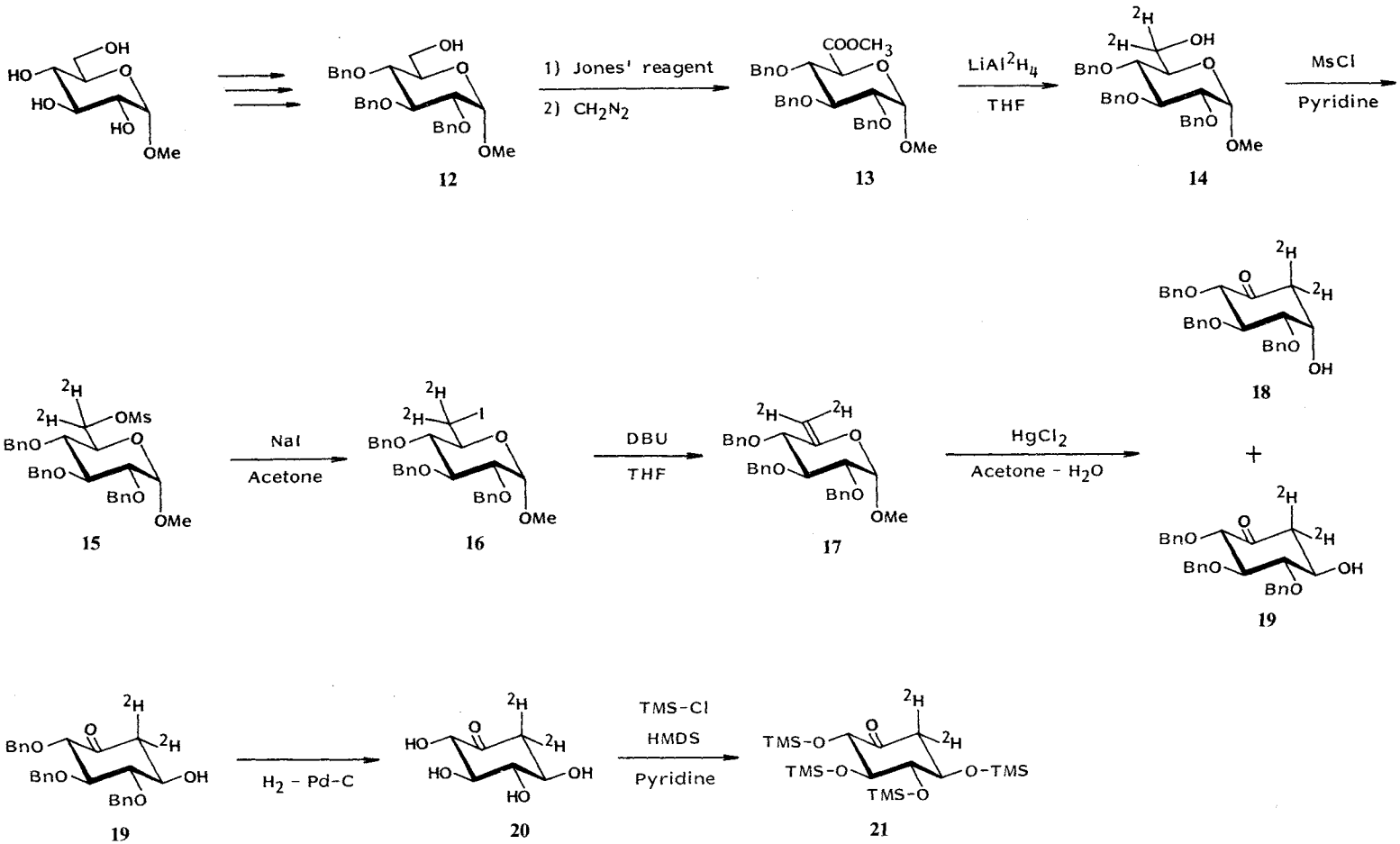
Synthesis of 2-deoxy-*scyllo*-inosose suitably labeled in such a way that the molecular weight is significantly different from the non-labeled counterpart was the second problem. It seems difficult to substitute two hydrogens by deuterons in a single conversion during the above-mentioned synthetic route for 2-deoxy-*scyllo*-inosose. Thus, we needed to employ other methods.

The Ferrier reaction involves conversion of a protected *xylo*-hex-5-enopyranoside to a 2-deoxyinosose

Scheme 1. Synthesis of 2-deoxy-*scyllo*-inosose.



Scheme 2. Synthesis of [2,2-$^2H_2$]-2-deoxy-*scyllo*-insose.



derivative by treatment with a Hg(II) salt in refluxing aqueous acetone as shown in the Scheme 2. Thus, the Ferrier reaction using the *xylo*-hex-5-enopyranoside dideuterated at C-6 may afford the dideuterated 2-deoxyinososse. The mass difference between the labeled and unlabeled specimen is 2 mass units, which seems appropriate for the present purpose. One drawback of the Ferrier reaction is that the hydroxyl group newly formed turns out to be axially oriented.[18] It was reported previously, however, that a methyl 2,3,4-tri-*O*-benzyl-*xylo*-hex-5-enopyranoside could give a mixture of isomers and the desired *scyllo*-isomer can be formed as a minor product.[19] Despite this known drawback, the Ferrier reaction seemed to be operationally straightforward and the required 6,6-dideuterio derivative of the precursor hexopyranoside (**14**) was known to be easily accessible in quantity from methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **12**[20] *via* the $LiAl^2H_4$ reduction of an intermediary 6-uronic acid ester (**13**).

From methyl 2,3,4-tri-*O*-benzyl-α-D-[6,6-$^2H_2$]glucopyranoside (**14**), methyl 2,3,4-tri-*O*-benzyl-5,6-didehydro-6-deoxy-α-D-[6,6-$^2H_2$]glucopyranoside (**17**) was prepared according to the reported method.[19,21] This compound **17** was then subjected to the Ferrier reaction conditions ($HgCl_2$, acetone - water, reflux), and a major *myo*-isomer **18** and the minor desired *scyllo*-derivatives **19** were obtained in a ratio of 3 : 1. The latter was isolated by conventional column chromatography and then deprotected to yield [2,2-$^2H_2$]-2-deoxy-*scyllo*-inososse **20**, which was further converted to the TMS ether (**21**). The ^1H NMR spectrum of **21** showed essentially no signals at δ 2.45 and 2.57. The deuterium enrichment of **20** was thus estimated to be >95%. Although the total yield of **20** was not significantly high, the desired [2,2-$^2H_2$]-2-deoxy-*scyllo*-inososse was successfully prepared.

It seems worthwhile to note that, while the stereochemical course of the biological 2-deoxy-*scyllo*-inososse formation in the biosynthesis of 2-deoxystreptamine is established, the steric course of the Ferrier reaction, especially about the prochirality at the C-6 position, is not known.

Experimental

MP's were determined by a Yanagimoto BY-1 melting point apparatus and are uncorrected. IR spectra were recorded on a Jasco IR-810 IR spectrometer. ^1H NMR spectra were recorded on a Jeol FX-200 (200 MHz) or a Jeol GSX-500 (500 MHz) spectrometer using, unless otherwise stated, TMS as an internal chemical shift standard in $CDCl_3$ solution. ^{13}C NMR spectra were measured with a Jeol FX-200 (50 MHz) or a Jeol GSX-500 (125 MHz) spectrometer, and the chemical shifts were calculated, unless otherwise stated, from the central signal of the $CDCl_3$ solvent at 77.0 ppm. GC-MS spectra were obtained by a Shimadzu GC-MS 9020DF mass spectrometer. TLC on Merck precoated plates Kieselgel 60 F_{254} Art. No. 5715 (0.25 mm thickness) was used for monitoring the reaction. Silica gel column chromatography was performed with Merck Kieselgel 60 (70~230 mesh ASTM).

Synthesis of 2-Deoxy-*scyllo*-inososse

1,2-*O*-Isopropylidene-*myo*-inositol (**3**)

Compound **3** was prepared according to the GIGG's method, MP 181~183°C (literature[7] 183~184°C).

1,2-*O*-Isopropylidene-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**4**)

To a solution of **3** (9.4 g, 42.7 mmol) in 100 ml of DMSO was added powdered NaOH (15.0 g, 375 mmol) and benzyl chloride (40 ml, d=1.10, 348 mmol), and the mixture was stirred vigorously for 3 hours at room temperature. While cooling in an iced water bath, water was added to the reaction mixture, which was then extracted with ether (300 ml × 3). The organic layer was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure to give an oily residue, which was chromatographed over silica gel (250 g, hexane - EtOAc, 4 : 1) to yield 24.5 g of **4** (99.0% yield): IR ($CHCl_3$) cm^{-1} 3020, 2900, 1440, 1125 and 1070; ^1H NMR

(200 MHz) δ 1.35 (3H, s), 1.52 (3H, s), 3.42 (1H, dd, J=8.8 and 10.7 Hz), 3.69 (1H, dd, J=3.6 and 8.8 Hz), 3.79 (1H, dd, J=8.8 and 9.1 Hz), 3.95 (1H, dd, J=9.1 and 10.7 Hz), 4.04 (1H, dd, J=5.7 and 8.8 Hz), 4.26 (1H, dd, J=3.6 and 5.7 Hz), 4.78 (8H, m, PhCH_2O–), and 7.32 (aromatic);

Anal Calcd for $C_{37}H_{40}O_6$: C 76.51, H 6.95.
Found: C 76.69, H 7.10.

3,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (**5**)

To a solution of 2.97 g of **4** (5.11 mmol) in 50 ml of MeOH was added 10 ml of 2 M-HCl. The mixture was warmed to 80°C (bath temp) for 30 minutes, then cooled to room temperature, and then neutralized with aq 5% KOH. The whole mixture was extracted with ether (100 ml × 3). The organic layer was washed with brine and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent, followed by recrystallization (hexane-ether) afforded 2.55 g of **5** (87.9% yield): MP 109~110°C; IR ($CHCl_3$) cm^{-1} 3560, 3020, 2900, 1455, 1135 and 1070; ^1H NMR (200 MHz) δ 2.46 (1H, br, –OH), 2.51 (1H, br, –OH), 3.46 (1H, t, J=9.3 Hz), 3.50 (2H, m), 3.81 (1H, t, J=10.1 Hz), 3.95 (1H, t, J=10.1 Hz), 4.20 (1H, t, J=2.8 Hz), 4.90 (8H, m, PhCH_2O–) and 7.30 (aromatic);

Anal Calcd for $C_{34}H_{36}O_6$: C 75.52, H 6.72.
Found: C 75.62, H 6.90.

Further recrystallization of the mother liquor gave 0.15 g of the 2nd crop (overall 97.8% yield).

1,2-Di-*O*-methanesulfonyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**6**)

To a solution of **5** (1.73 g, 3.19 mmol) in 10 ml of pyridine was added 1.0 ml of methanesulfonyl chloride (MsCl, 1.48 g, 12.9 mmol) and 100 mg of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred at room temperature for 15 hours. Additional 1 ml of MsCl was added to the mixture and stirring was continued for 4 hours. The mixture was then diluted with water, and extracted with ether (100 ml × 3). The organic layer was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane-EtOAc, 3:1) to afford 1.91 g of **6** (86.0% yield): MP 147~149°C (recrystallization from hexane); IR ($CHCl_3$) cm^{-1} 3040, 2875, 1455, 1360, 1185, 1095, 1070 and 925; ^1H NMR (200 MHz) δ 3.01 (3H, s), 3.08 (3H, s), 3.52 (2H, m), 3.86 (1H, t, J=9.7 Hz), 3.94 (1H, t, J=9.3 Hz), 4.51 (1H, dd, J=2.7 and 9.3 Hz), 4.93 (8H, m, PhCH_2O–), 5.49 (1H, t, J=2.7 Hz) and 7.30 (aromatic);

Anal Calcd for $C_{36}H_{40}O_{10}S_2$: C 62.03, H 5.79, S 9.21.
Found: C 62.04, H 5.85, S 9.22.

3,5/4,6-Tetrabenzyloxycyclohexene (**7**)

To a solution of **6** (1.14 g, 16.4 mmol) in 15 ml of DMF was added 4.0 g of NaI and 2.0 g of activated Zn dust. The mixture was heated at reflux for 2 hours, and then cooled to room temperature. The resulting mixture was diluted with hexane and water, and filtered. The filtrate was extracted with ether (30 ml × 3). The combined organic layer was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent yielded 732 mg of **7** (88.3% yield): MP 76~79°C; IR ($CHCl_3$) cm^{-1} 3000, 2860, 1500, 1455, 1065 and 1025; ^1H NMR (200 MHz) δ 3.73 (2H, q), 4.22 (2H, q), 4.66, 4.72 (4H, d, J=11.4 Hz), 4.85, 4.93 (4H, d, J=10.3 Hz) and 7.30 (aromatic);

Anal Calcd for $C_{34}H_{34}O_4$: C 80.59, H 6.76.
Found: C 80.30, H 6.84.

3,5/4,6-Tetrabenzyloxycyclohexene-1,2-oxide (**8**)

To a solution of **7** (594 mg, 1.17 mmol) in 5 ml of CH_2Cl_2 was added *m*-CPBA (85%, 319 mg). The mixture was stirred for 15 hours at room temperature. To the mixture were added ether and water, the layers were separated. The organic layer was washed with 1% Na_2CO_3 soln and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent afforded 612 mg of a residue. Further purification was performed with silica gel (50 g, hexane-EtOAc, 7:1~5:1) to yield 268 mg of **8** (43.6% yield): MP 54~57°C; IR ($CHCl_3$) cm^{-1} 3000, 2900, 2870, 1455 and 1065; ^1H NMR (200 MHz) δ 3.21 (1H, d, J=4.1 Hz), 3.33 (1H, m), 3.46 (1H, dd, J=8.0 and 10.0 Hz), 3.64 (1H, dd, J=8.6 and 10.0 Hz), 3.89 (1H, d, J=8.6 Hz),

3.91 (1H, d, J=8.0 Hz), 4.74 (4H, d, J=10.3 Hz), 4.83 (4H, d, J=9.0 Hz) and 7.38 (aromatic);

Anal Calcd for $C_{34}H_{34}O_5$: C 78.12, H 6.56.
Found: C 78.30, H 6.61.

With further elution, 246 mg of **7** (41.5%) was recovered.

2,4/3,5-Tetrabenzyloxycyclohexanol (**9**)

To a solution of **8** (1.34 g, 2.56 mmol) in 20 ml of THF in an iced water bath was added 154 mg of $LiAlH_4$. The mixture was warmed to room temperature and stirred for 1.5 hours. After being cooled in an iced water bath, the mixture was diluted with ether, and then sodium potassium tartarate was added. The layers were separated and the aqueous layer was further extracted with ether (50 ml × 2). The combined organic extract was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent yielded 1.104 g of crude **9**. Purification with column chromatography (50 g, hexane - EtOAc, 1 : 1) afforded 1.07 g (79.2% yield) of **9**: IR ($CHCl_3$) cm^{-1} 3560, 3050, 2960, 1460, 1075 and 1030; ^1H NMR (200 MHz) δ 1.38 (1H, dd, J=11.4 and 14.1 Hz), 2.36 (1H, td, J=4.0, 14.1 Hz), 2.43 (1H, br s), 3.45 (2H, m), 3.83 (1H, t, J=9.4 Hz), 3.90 (1H, ddd, J=4.0, 11.4 and 14.0 Hz), 4.10 (1H, br), 4.68 (4H, m, PhCH_2O–), 4.86 (4H, m, PhCH_2O–) and 7.36 (aromatic);

Anal Calcd for $C_{34}H_{36}O_2$: C 77.82, H 6.92.
Found: C 77.55, H 6.93.

2,4/3,5-Tetrabenzyloxycyclohexanone (**10**)

To a solution of **9** (391 mg, 0.75 mmol) in 6 ml of CH_2Cl_2 was added powdered molecular sieves 3A (1.2 g) and 513 mg of pyridinium chlorochromate (PCC). With vigorous stirring, the reaction was completed in 15 minutes. The reaction mixture was diluted with ether and passed through a Florisil column, which was further eluted with ether. The eluate was evaporated under reduced pressure to give 338 mg (86.7% yield) of **10**: MP 79 ~ 81°C; IR ($CHCl_3$) cm^{-1} 3050, 1735, 1460 and 1075; ^1H NMR (200 MHz) δ 2.54 (1H, dd, J=10.4 and 13.1 Hz), 2.81 (1H, dd, J=4.9 and 13.1 Hz), 3.65 (2H, m), 3.92 (1H, d, J=8.3 Hz), 4.24 (1H, d, J=10.4 Hz), 4.76 (8H, m, PhCH_2O–) and 7.35 (aromatic);

Anal Calcd for $C_{34}H_{34}O_5$: C 78.12, H 6.56.
Found: C 77.85, H 6.75.

2-Deoxy-*scyllo*-inosose (**1**)

To a solution of **10** (155 mg, 0.30 mmol) in MeOH - H_2O - AcOH (8 ml : 2 ml : 2 ml) was added 100 mg of 10% Pd - C (Kawaken Fine Chemicals Co. Ltd.). The mixture was degassed by an water-aspilator and then refilled with H_2 gas. The mixture was stirred vigorously overnight under a H_2 blanket. The reaction was diluted with water. The catalyst was removed by filtration with an aid of Celite 545. The filtrate was evaporated at 40°C to yield 59.8 mg of syrupy **1**: ^1H NMR (200 MHz, D_2O: $\delta_{HDO=4.75\ ppm}$) δ 1.52 (1H, t, J=12.9 Hz), 2.01 (1H, dd, J=5.4 and 12.9 Hz), 2.58 (1H, d), 3.30 (1H, m), 3.56 (1H, m), 4.19 (1H, d, J=12.9 Hz); ^{13}C NMR (D_2O: $\delta_{dioxane=67.4\ ppm}$) a keto-form δ 45.06, 69.06, 74.72, 76.91, 77.84 and 207.59; a hydrate form δ 41.55, 69.32, 74.40, 77.11, 77.84 and 94.09.

Tetra-*O*-trimethylsilyl-2-deoxy-*scyllo*-inosose (**11**)

Deprotection of **10** (538 mg, 1.03 mmol) was carried out as described above in MeOH - H_2O - AcOH (16 ml : 4 ml : 2 ml). Careful evaporation of the resulting mixture afforded 206 mg of unpurified **1** as a solid, which was re-suspended in 5 ml of pyridine. To this mixture was added 1 ml of chlorotrimethylsilane and 1 ml of hexamethyldisilazane, and stirring was continued for 1 hour at room temperature. Additional 1 ml of chrolotrimethylsilane was added and stirring was further continued for 30 minutes. The reaction mixture was diluted with ether, and water was added. The organic layer was separated and the aqueous layer was extracted with ether (20 ml × 3). The combined organic extract was washed successively with 0.1 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified with a silica gel column (hexane - EtOAc, 10 : 1) to yield 206 mg of **11** (44.2% isolated yield for 2 steps): EI-MS: m/z 450 (M^+), 435, 360 (M − TMSOH), 305, 270 (M − 2 × TMSOH), 147 and 73 (base peak); IR ($CHCl_3$) cm^{-1} 2975, 1740, 1260, 1080, 905, 875 and 855; ^1H NMR (500 MHz) δ −0.3 ~ 0.2 (m), 2.45 (1H, dd, J=10.9 and 13.4 Hz), 2.57 (1H, dd, J=4.8 and 13.4 Hz),

3.38 (1H, dd, J=7.8 and 9.9 Hz), 3.57 (1H, ddd, J=4.8, 8.7 and 13.4 Hz), 3.66 (1H, dd, J=7.8 and 8.8 Hz) and 4.13 (1H, d, J=9.9 Hz); ^{13}C NMR (125 MHz) δ 0.0~1.0 (m), 45.81, 70.01, 78.02, 78.47, 79.78 and 204.73;

Anal Calcd for $C_{18}H_{42}O_5Si_4$: C 47.98, H 9.40.
Found: C 48.20, H 9.26.

Synthesis of [2,2-$^2H_2$]-2-Deoxy-*scyllo*-inosose

Methyl 1-*O*-Methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranuronate (**13**)

To a cooled solution of 2.598 g (5.60 mmol) of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **12**,[20] in 15 ml of MeOH in an iced water bath was added 0.5 ml of a JONES' reagent (2.74 mmol Cr^{3+}/liter aqueous sulfuric acid solution). The mixture was stirred for 30 minutes, and then 2-PrOH was added until the solution color turned to green. This mixture was diluted with water and extracted with ether. The organic layer was washed with brine and dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent yielded 2.732 g of a residue, which was directly treated with ethereal CH_2N_2. After removal of solvent, the product was purified by silica gel column chromatography (50 g, hexane - EtOAc, 5 : 1 ~ 3 : 1) to yield 1.682 g of **13** (68.1%) as a syrup: $[\alpha]_D$ +11.2° (c 0.64, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 2985, 2920, 1735, 1430, 1350, 1060 and 900; ^{1}H NMR (200 MHz) δ 3.40 (3H, s), 3.57 (1H, dd, J=3.4 and 9.5 Hz), 3.70 (3H, s, $-COOCH_3$), 3.71 (1H, dd, J=9.0 and 10.0 Hz), 3.99 (1H, t, J=9.4 Hz), 4.25 (1H, d, J=10.0 Hz), 4.59 (1H, d, J=3.4 Hz), 4.50~5.00 (6H, PhCH_2O–) and 7.27~7.40 (aromatic).

Anal Calcd for $C_{29}H_{32}O_7$: C 70.72, H 6.55.
Found: C 71.00, H 6.35.

Methyl [6,6-$^2H_2$]-2,3,4-Tri-*O*-benzyl-α-D-glucopyranoside (**14**)

To a solution of **13** (1.535 g, 3.11 mmol) in 15 ml of THF was added 193 mg of $LiAl^2H_4$ (Merck, 99 atom% enriched) with cooling in an iced water bath. The mixture was allowed to warm up to room temperature and stirred for 15 minutes. After being diluted with ether, the mixture was cooled in an iced water bath, and sodium potassium tartarate was added. The layers were separated and the aqueous layer was further extracted with ether (50 ml × 2). The combined organic extract was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. Filtration and evaporation of the solvent yielded 1.336 g of **14** (91.1% yield) as a syrup: $[\alpha]_D$ +22.2° (c 0.71, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3000, 2910, 1445, 1350, 1145 and 1060; ^{1}H NMR (200 MHz) δ 3.36 (3H, s, $-OCH_3$), 3.51 (1H, dd, J=3.6 and 9.7 Hz), 3.61 (1H, t, J=8.6 Hz), 4.00 (1H, dd, J=8.6 and 9.7 Hz), 4.56 (1H, d, J=3.6 Hz), 4.50~5.00 (6H, PhCH_2O–) and 7.27~7.40 (aromatic).

Anal Calcd for $C_{28}H_{30}{}^2H_2O_6$: C 72.08, H+^{2}H 6.91.
Found: C 72.30, H+^{2}H 6.81.

Methyl [6,6-$^2H_2$]-2,3,4-Tri-*O*-benzyl-6-*O*-methanesulfonyl-α-D-glucopyranoside (**15**)

To a solution of **14** (1.316 g, 2.80 mmol) in 15 ml of pyridine was added 0.5 ml of MsCl (0.74 g, 6.5 mmol) and the reaction mixture was stirred at room temperature for 30 minutes. The mixture was then diluted with water, and extracted with ether. The organic layer was washed successively with 2 M-HCl, satd $NaHCO_3$, and brine, and then dried over anhydrous $MgSO_4$. After filtration and removal of the solvent, the residue was purified by silica gel column chromatography (hexane - EtOAc, 3 : 1 ~ 2 : 1) to afford 1.488 g of **15** (96.8% yield): MP 72~74°C; $[\alpha]_D$ +25.2° (c 0.49, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 2970, 2920, 1455, 1360, 1175, 1070 and 980; ^{1}H NMR (200 MHz) δ 2.97 (3H, s), 3.38 (3H, s), 3.48 (1H, t, J=9.6 Hz), 3.51 (1H, dd, J=3.4 and 9.7 Hz), 3.83 (1H, d, J=10.2 Hz), 4.01 (1H, t, J=9.6 Hz), 4.58 (1H, d, J=3.4 Hz), 4.58~5.03 (8H, PhCH_2O–) and 7.30 (aromatic).

Anal Calcd for $C_{29}H_{32}{}^2H_2O_8S$: C 63.95, H+^{2}H 6.29.
Found: C 63.75, H+^{2}H 6.45.

Methyl [6,6-$^2H_2$]-2,3,4-Tri-*O*-benzyl-6-deoxy-6-iodo-α-D-glucopyranoside (**16**)

To a solution of **15** (1.455 g, 2.67 mmol) in 25 ml of acetone was added 2.5 g of NaI, and the mixture was heated at reflux for 24 hours. The resulting mixture was cooled to room temperature, and diluted with ether. This mixture was treated with a Na_2SO_3 solution and the whole was extracted with ether. The

combined organic extract was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified with silica gel (50 g, hexane - EtOAc, 4 : 1 ~ 2 : 1) to afford 1.347 g of **16** (87.4% yield) as a syrup: $[\alpha]_D$ +30.7° (*c* 0.58, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3000, 2850, 1490, 1445, 1375, 1065; ^1H NMR (200 MHz) δ 3.33 (1H, t, J=9.5 Hz), 3.41 (3H, s), 3.42 (1H, t, J=8.5 Hz), 3.53 (1H, dd, J=3.5 and 9.5 Hz), 4.00 (1H, dd, J=8.5 and 9.4 Hz), 4.60 (1H, d, J=3.6 Hz), 4.62 ~ 5.10 (8H, PhCH_2O–) and 7.30 (aromatic).

Anal Calcd for $C_{28}H_{29}{}^2H_2O_5I$: C 58.34, H + ^{2}H 5.42.
Found: C 58.09, H + ^{2}H 5.47.

Methyl [6,6-$^2H_2$]-2,3,4-Tri-*O*-benzyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside (**17**)

To a solution of **16** (1.347 g, 2.33 mmol) in 30 ml of THF was added 1.7 ml of 1,8-diazabicyclo[5,4,0]undec-7-ene (d = 1.019, 1.73 g, 11.4 mmol), and the mixture was heated at reflux for 24 hours in a N_2 atmosphere. The resulting mixture was cooled to room temperature, diluted with water and extracted with ether. The combined organic extract was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified with silica gel (25 g, hexane - EtOAc, 10 : 1 ~ 7 : 1) to afforded 458 mg of **17** (43.8% yield): MP 56 ~ 59°C; $[\alpha]_D$ −36.1° (*c* 0.41, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3000, 2985, 1630, 1490, 1445, 1365 and 1070; ^1H NMR (200 MHz) δ 3.38 (1H, t, J=9.5 Hz), 3.60 (1H, ddd, J=1.0, 3.4 and 8.6 Hz), 3.89 (1H, dd, J=9.0 and 10.0 Hz), 3.94 (1H, d, J=10.0 Hz), 4.61 (1H, d, J=3.4 Hz), 4.70 ~ 4.97 (8H, PhCH_2O–) and 7.30 (aromatic).

Anal Calcd for $C_{28}H_{28}{}^2H_2O_5$: C 74.98, H + ^{2}H 6.74.
Found: C 75.25, H + ^{2}H 6.51.

(2*S*,3*R*,4*S*,5*S*)-[6,6-$^2H_2$]-2,3,4-Tribenzyloxy-5-hydroxycyclohexanone (**18**) and (2*S*,3*R*,4*S*,5*R*)-[6,6-$^2H_2$]-2,3,4-Tribenzyloxy-5-hydroxycyclohexanone (**19**)

To a solution of **17** (91.4 mg, 0.20 mmol) in a mixture of 4 ml of acetone and 2 ml of water was added 107 mg of $HgCl_2$, and the mixture was heated at reflux for 1 hour. The reaction mixture was cooled to room temperature, diluted with water and then extracted with EtOAc. The combined organic extract was washed successively with 2 M-HCl, satd $NaHCO_3$ and brine, and then dried over anhydrous $MgSO_4$. After filtration and evaporation of the solvent, the residue was purified with silica gel (10 g, hexane - EtOAc, 2 : 1) to afford 11.4 mg of **19** (12.9% yield): MP 125 ~ 128°C; $[\alpha]_D$ −38.9° (*c* 0.69, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3550, 3000, 2850, 1730, 1450, 1075; ^1H NMR (200 MHz) δ 2.51 (1H, br), 3.76 (1H, dd, J=2.4 and 3.1 Hz), 3.60 (1H, d, J=2.4 Hz), 4.04 (1H, s), 4.22 (1H, br d, J=3.1 Hz), 4.50 ~ 4.90 (6H, PhCH_2O–), and 7.30 (aromatic).

Anal Calcd for $C_{27}H_{26}{}^2H_2O_5$: C 74.63, H + ^{2}H 6.49.
Found: C 74.56, H + ^{2}H 6.63.

Further elution with hexane - EtOAc (1 : 1) afforded 38.4 mg of **18** (43.5% yield): MP 111 ~ 113°C; $[\alpha]_D$ −43.6° (*c* 0.45, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 3550, 3000, 2850, 1730, 1450 and 1075; ^1H NMR (200 MHz) δ 2.42 (1H, br), 3.69 (3H, br), 4.16 (1H, m), 4.51 (1H, d, J=11.5 Hz), 4.69 (1H, d, J=10.5 Hz), 4.75 (1H, d, J=11.0 Hz), 4.93 (1H, d, J=10.5 Hz), 4.95 (1H, d, J=11.0 Hz), 5.03 (1H, d, J=11.5 Hz) and 7.30 (aromatic).

Anal Calcd for $C_{27}H_{26}{}^2H_2O_5$: C 74.63, H + ^{2}H 6.49.
Found: C 74.91, H + ^{2}H 6.68.

Tetra-*O*-trimethylsilyl-[2,2-$^2H_2$]-2-deoxy-*scyllo*-inosose (**21**)

By a similar procedure to the preparation of **11**, the protected [2,2-$^2H_2$]-2-deoxy-*scyllo*-inosose (**19**) was converted to **21**.

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