

Total Synthesis of (–)-Tetrodotoxin from D-Glucose: A New Route to Multi-Functionalized Cyclitol Employing the Ferrier(II) Reaction toward (–)-Tetrodotoxin

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Total synthesis of (–)-tetrodotoxin (TTX) from D-glucose is described. As a critical transformation step for synthesizing TTX, a key multi-functionalized cyclitol was prepared from D-glucose employing the Ferrier(II) reaction. Stereospecific introduction of three functionalized branched chains were achieved via Peterson olefination and spiro α -chloroepoxidation of the corresponding carbonyl derivatives.

The structure of tetrodotoxin (TTX), a toxic principle in puffer fish poisoning, was reported in 1964 by four separate research groups: Tsuda's,^{1a} Hirata's,^{1b} Woodward's,^{1c} and Mosher's^{1d} groups. TTX is a useful biological tool in neurophysiology due to its specificity for blocking voltage-gated Na⁺ ion channels in nervous systems.² Recently, TTX and several analogs have been found and isolated (Figure 1), not only from aquatic organisms such as puffer fish, but also from terrestrial animals.^{3,4} TTX and its analogs can provide important insights into pharmaceutical studies, elucidation of structure–activity relationships, and biological roles.⁵ Accordingly, the ability to synthesize larger amounts of TTX and its analogs has grown in importance, however still remains as an extremely difficult, yet fascinating, challenge.⁶ In 2003, the Isobe and Du Bois groups both succeeded in the synthesis of (–)-TTX.^{7a,7d,7e} In the following year, Isobe's group reported

an improved route with fewer steps.^{7b,7c,7f} We also achieved the total synthesis of (±)-TTX from *myo*-inositol in 2005,^{8a} and (–)-TTX from D-glucose employing the Henry reaction (intramolecular nitro aldol reaction) in 2008.^{8b} Moreover, recently Noheda filed a patent application for a synthetic method for TTX, analogs, and its intermediates.⁹

In this area, a practical synthetic route for TTX and its analogs to satisfy biological and pharmacological needs has been increasingly in demand. Particularly, development of a convergent synthetic method to prepare the multi-functionalized cyclitol of the TTX skeleton rather than a linear protocol is required. Herein, we describe our success in another convergent new route for synthesizing (–)-TTX from D-glucose via the multi-functionalized cyclitol **10a** employing the Ferrier(II) reaction¹⁰ as the key skeleton construction. Our synthetic plan was based on our prior experiences in the total synthesis of (±)-TTX from *myo*-inositol^{8a} (Scheme 1). Both total syntheses were successfully carried out using the common intermediate **D**, with the later work accomplished in an enantiospecific manner. Compound **D** was derived from D-glucose in the following manner. Compound **D** can be synthesized from multi-functionalized cyclitol **F**,⁸ which can be prepared by the reaction of enol acetate **G** employing the Ferrier(II) reaction.¹⁰ Compound **G** can be obtained by standard transformations of D-glucose by using stereoselective introduction of the branched chain at the C-3 of D-glucose (at the C-6 of TTX). The transformation of **D** into (–)-TTX, via compound **C**, **B**, and **A**, were accomplished in a similar manner as reported in the previous paper.⁸

Results and Discussion

The total synthesis of (–)-TTX from D-glucose is shown in Scheme 2. Methyl 2-*O*-benzyl-4,6-*O*-benzylidene- α -D-glucopyranosid-3-ulose (**1**) was prepared by regioselective benzylation¹¹ followed by the Swern oxidation of methyl 4,6-*O*-

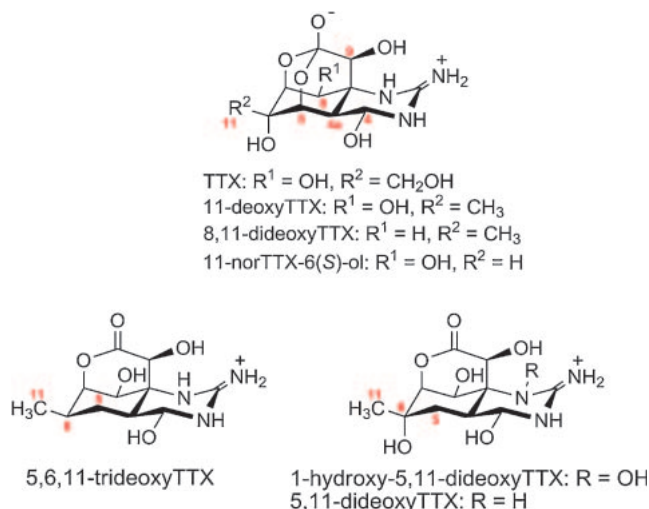
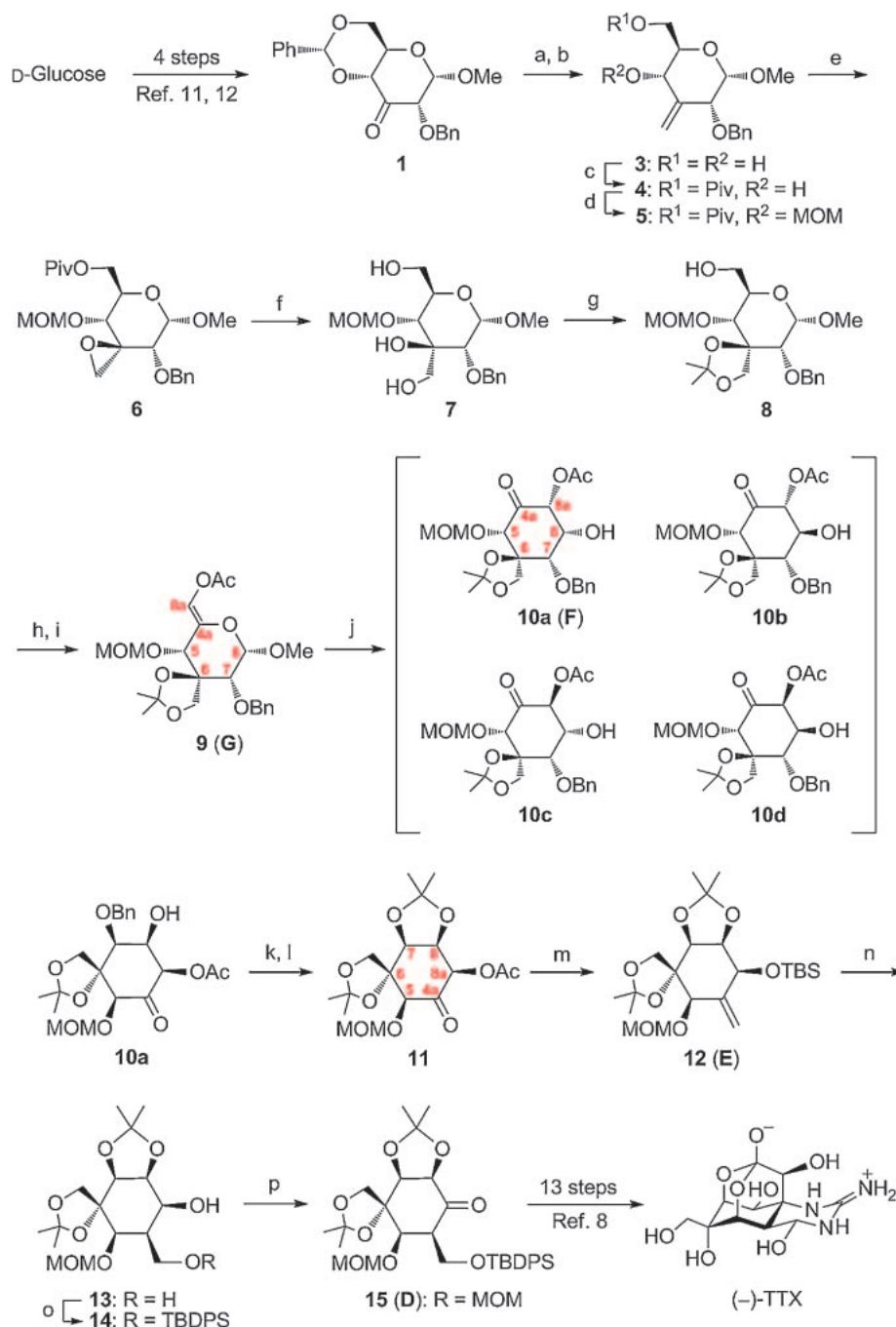


Figure 1.¹⁸ Tetrodotoxin and its analogs.^{1,3}



Scheme 2.¹⁸ Total synthesis of (-)-TTX from D-glucose. Reagents and conditions: a) TMSCH₂MgCl, Et₂O–CH₂Cl₂ (94%); b) 60% AcOH aq (99%); c) PivCl, Py., CH₂Cl₂ (94%); d) P₂O₅, CH₂(OCH₃)₂, CH₂Cl₂, (82%); e) *m*-CPBA, (CH₂Cl)₂ (75%); f) *n*-Bu₄NOH, THF (83%); g) (CH₃)₂C(OCH₃)₂, *p*-TsOH, CH₂Cl₂ (75%); h) TFAA, DMSO, Et₃N, CH₂Cl₂; i) K₂CO₃, Ac₂O, CH₃CN (72%, 2 steps); j) Hg(OAc)₂, AcOH aq–acetone, then NaCl aq (58%); k) 20% Pd(OH)₂–C, H₂, EtOH (quant.); l) (CH₃)₂C(OCH₃)₂, *p*-TsOH, acetone–CH₂Cl₂ (66%); m) [i] TMSCH₂MgCl, Et₂O (56%); [ii] TBS–Cl, imidazole, CH₂Cl₂ (88%); n) [i] BH₃·THF, THF, then 10% NaOH aq, 30% H₂O₂ aq; [ii] *n*-Bu₄NF, THF (74%, 2 steps); o) *tert*-butyldiphenylsilyl chloride (TBDPS–Cl), imidazole, CH₂Cl₂ (92%); p) Dess–Martin periodinane, CH₂Cl₂ (99%).

Conclusion

The total synthesis of (-)-TTX from D-glucose featuring the Ferrier(II) reaction as the key skeleton construction was successfully carried out using effective and stereoselective steps. In studies of TTX synthesis, our group has achieved the

total synthesis of TTX in three routes.⁸ The proper selection of these synthetic methodologies may be useful for not only compounds related to TTX (including enabled derivatives), but also other highly complex multi-functionalized cyclitols bearing branched chain structures such as cyclophellitol, mytilitol, and laminitol.¹⁷

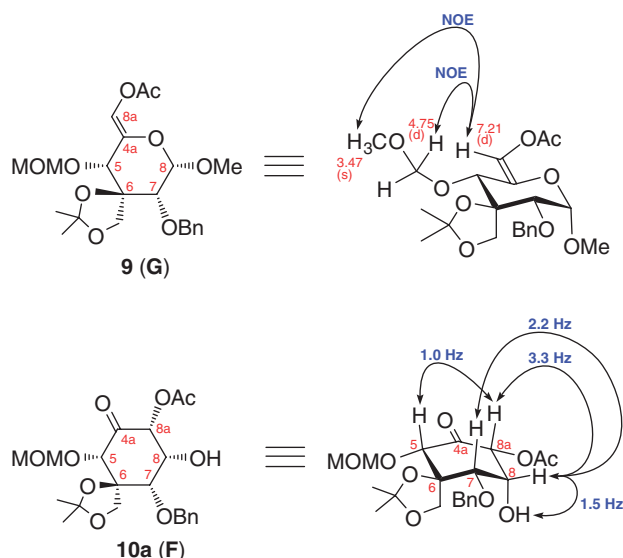


Figure 2.¹⁸ Structure assignments of enol acetate **9** and cyclitol **10a** (Chemical shifts, split pattern, NOE correlation of **9**, and J values of **10a** with the corresponding TTX numbering).

Table 1. The Ferrier(II) Reaction Conditions and Products Yields

Entry	Reagent	Time /h	Ratio ^{a)} 10a:10b:10c:10d	Isolated yield of 10a /%
1	Hg(OCOCH ₃) ₂	0.3	6:0:1:3	33
2	HgCl ₂	4	0:0:1:2	trace
3	HgO	5	5:0:2:1	48
4	Hg(OAc) ₂	1.5	6:2:1:0	58

a) Determined by ¹H NMR spectrum.

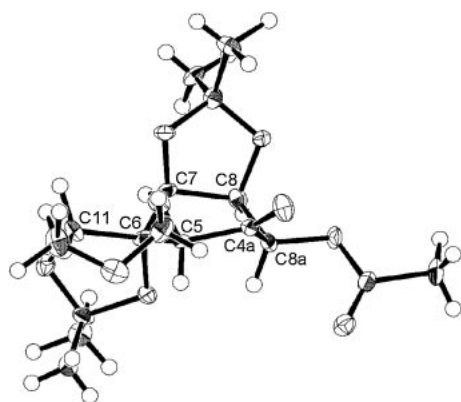


Figure 3.¹⁸ ORTEP drawing of **11** with the corresponding TTX numbering. Thermal ellipsoids were scaled to enclose 30% probability.

Experimental

Melting points were measured using a Yanaco Model MP-J3 micro-melting point apparatus, and are uncorrected. IR spectra were recorded using a SHIMADZU IR Prestige-21 spectrometer in KBr. ¹H and ¹³C NMR spectra were measured with JEOL JNM-ECA500 and -600 spectrometers in CDCl₃ or 3% CD₃COOD/D₂O solution with tetramethylsilane used as internal standard. Specific

rotations were measured in 0.5 dm tubes using a JASCO P-1020 polarimeter in CHCl₃ or 3% CD₃COOD/D₂O. Mass spectra were obtained on a SHIMADZU JMS-T100CS. X-ray diffraction was measurement by Rigaku Saturn 70 diffractometer with graphite monochromated Mo K α radiation. Structure solution and refinement were performed using CrystalStructure 3.8. Crystallographic data have been deposited with Cambridge Crystallographic Data Centre: Deposition number CCDC-644815 for compound No. 11. Copies of the data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, U.K.; Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Methyl 2-O-Benzyl-4,6-O-benzylidene- α -D-glucopyranoside-3-ulose (1). To a stirred solution of trifluoroacetic anhydride (1.12 mL, 8.07 mmol) in dry CH₂Cl₂ (10 mL), dimethyl sulfoxide (0.96 mL, 13.5 mmol) was added at -78°C under argon. After 30 min, a solution of methyl 2-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside¹⁰ (1.00 g, 2.69 mmol) in dry CH₂Cl₂ (5 mL) was added dropwise to the reaction mixture at -78°C . After 15 min, triethylamine (2.61 mL, 18.8 mmol) was added dropwise to the above mixture and stirred for 5 min. After the disappearance of 2-O-benzylglucopyranoside on TLC with toluene–acetone (8:1 v/v), the reaction mixture was poured into saturated aq NaHCO₃ solution, extracted with CHCl₃, washed with brine and water, dried over anhyd MgSO₄, and evaporated to give **1**, which was purified by recrystallization from hexane–EtOAc; mp 177.0 – 178.0°C (colorless needles, hexane–EtOAc); R_f = 0.38 (toluene–acetone = 8:1 v/v); $[\alpha]_D^{25}$ -32.4° (c 0.73, CHCl₃); IR (KBr, disk): ν 1748 cm⁻¹ (C=O); ¹H NMR (600 MHz): δ 7.51–7.31 (10H, m, PhH), 5.53 (1H, s, PhCH), 5.06 (1H, d, $J_{2,1}$ = 4.1 Hz, H-1), 4.97, 4.60 (2H, each d, $J_{A,B}$ = 12.2 Hz, $-\text{CH}_2\text{Ph}$), 4.39 (1H, dd, $J_{6\text{eq},6\text{ax}}$ = 10.2 Hz, $J_{6\text{eq},5}$ = 4.8 Hz, H-6eq), 4.20 (1H, dd, $J_{4,5}$ = 9.8 Hz, $J_{4,2}$ = 1.4 Hz, H-4), 4.14 (1H, dd, $J_{2,1}$ = 4.1 Hz, $J_{2,4}$ = 1.4 Hz, H-2), 4.09 (1H, ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6\text{eq}}$ = 4.8 Hz, $J_{5,6\text{ax}}$ = 10.4 Hz, H-5), 3.87 (1H, dd, $J_{6\text{ax},6\text{eq}}$ = 10.2 Hz, $J_{6\text{ax},5}$ = 10.4 Hz, H-6ax), 3.44 (3H, s, OCH₃). ¹³C NMR (150 MHz): δ 196.27, 136.9, 136.3, 129.3, 128.6, 128.2, 126.4, 102.5, 101.9, 82.1, 79.6, 72.6, 69.4, 65.4. Anal. Calcd for C₂₁H₂₂O₆ (370.40): C, 68.10; H, 5.99%. Found: C, 68.43; H, 6.33%.

Methyl 2-O-Benzyl-4,6-O-benzylidene-3-C-trimethylsilylmethyl- α -D-allopyranoside (2). To a solution of 3-ulose **1** (781 mg, 2.1 mmol) in a mixture of dry CH₂Cl₂–Et₂O (1:1 v/v, 70 mL), trimethylsilylmethylmagnesium chloride (0.45 M in Et₂O, 7.0 mL, 3.15 mmol) was added and stirred for 1 h under argon. After the disappearance of starting compound **1** on TLC with hexane–EtOAc (2:1 v/v), the mixture was poured into saturated aq NH₄Cl solution, extracted with EtOAc, washed with brine and water, dried over anhyd MgSO₄, evaporated to give **2** (913 mg, 94% yield), which was purified on a column of silica gel with hexane–EtOAc (4:1 v/v). colorless syrup; R_f = 0.25 (hexane–EtOAc = 4:1 v/v); $[\alpha]_D^{25}$ $+32.9^{\circ}$ (c 1.07, CHCl₃); IR (KBr, neat): ν 3511 cm⁻¹ (OH); ¹H NMR (600 MHz): δ 7.52–7.31 (10H, m, PhH), 5.48 (1H, s, PhCH), 4.73 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.68 (2H, s, $\text{CH}_2\text{-Ph}$), 4.32 (1H, dd, $J_{6\text{eq},6\text{ax}}$ = 10.3 Hz, $J_{6\text{eq},5}$ = 5.2 Hz, H-6eq), 4.10 (1H, ddd, $J_{5,6\text{eq}}$ = 5.2 Hz, $J_{5,6\text{ax}}$ = 10.3 Hz, $J_{5,4}$ = 9.2 Hz, H-5), 3.69 (1H, dd, $J_{6\text{ax},5}$ = 10.3 Hz, $J_{6\text{ax},6\text{eq}}$ = 10.3 Hz, H-6ax), 3.49 (1H, s, OH), 3.39 (3H, s, OCH₃), 3.38 (1H, d, $J_{2,1}$ = 3.4 Hz, H-2), 3.36 (1H, d, $J_{4,5}$ = 9.2 Hz, H-4), 1.36, 1.26 (2H, each d, $J_{A,B}$ = 14.9 Hz, $\text{CH}_2\text{Si}(\text{CH}_3)_3$), 0.00 (9H, s, $\text{CH}_2\text{Si}(\text{CH}_3)_3$). ¹³C NMR (150 MHz): δ 137.7, 137.3, 129.0, 128.5, 128.1, 128.0, 126.5, 102.2, 98.5, 82.6, 78.9, 74.9, 72.3, 69.1, 59.4, 55.7, 24.4,

0.39. NOE correlation: H-5-OH; H-3'-H-2, -H-4. Anal. Calcd for $C_{25}H_{34}O_6Si$ (458.62): C, 65.47; H, 7.47%. Found: C, 65.22; H, 7.28%.

Methyl 2-O-Benzyl-3-deoxy-3-methylene- α -D-ribo-hexopyranoside (3). Compound **2** (98.4 mg, 0.214 mmol) was dissolved in 60% aq acetic acid solution (3 mL), and stirred for 4 h at 70 °C. After the disappearance of starting compound **2** on TLC with hexane-EtOAc (4:1 v/v), the reaction mixture was evaporated to give olefin **3** (59.4 mg, 99% yield), which was purified on a column of silica gel with hexane-EtOAc (1:4 v/v). Colorless syrup; R_f = 0.55 (hexane-EtOAc = 1:4 v/v); $[\alpha]_D^{25} +21.1^\circ$ (*c* 1.45, $CHCl_3$); IR (KBr, neat): ν 3445 cm^{-1} (OH), 1661 cm^{-1} (C=C); 1H NMR (600 MHz): δ 7.38–7.29 (5H, m, PhH), 5.41–5.31 (2H, m, H-3'), 4.80, 4.59 (2H, each d, $J_{A,B}$ = 12.5 Hz, CH_2Ph), 4.71 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1), 4.07 (1H, dd, $J_{6a,5}$ = 7.4 Hz, $J_{6a,6b}$ = 9.3 Hz, H-6a), 3.93 (1H, m, H-2), 3.85 (2H, m, H-6b, H-4), 3.50 (1H, ddd, $J_{5,6a}$ = 7.4 Hz, $J_{5,6b}$ = 4.0 Hz, $J_{5,4}$ = 8.9 Hz, H-5), 3.40 (3H, s, OCH_3), 2.28 (1H, d, $J_{OH,4}$ = 7.2 Hz, 4-OH), 2.05 (1H, s, 6-OH). ^{13}C NMR (150 MHz): δ 143.5, 137.9, 128.5, 128.1, 127.9, 127.7, 105.3, 98.7, 97.7, 76.5, 73.7, 72.1, 68.6, 62.6, 55.3. Anal. Calcd for $C_{15}H_{20}O_5$ (280.32): C, 64.27; H, 7.19%. Found: C, 64.15; H, 7.16%.

Methyl 2-O-Benzyl-3-deoxy-3-methylene-6-O-pivaloyl- α -D-ribo-hexopyranoside (4). To a solution of olefin **3** (104 mg, 0.37 mmol) in dry CH_2Cl_2 (2.5 mL) and pyridine (0.5 mL), pivaloyl chloride (92 μ L, 0.74 mmol) was added and stirred at 0 °C. After 80 min, pivaloyl chloride (46 μ L, 0.37 mmol) was added to the above mixture at rt. After the disappearance of starting compound **3** on TLC with hexane-EtOAc (1:1 v/v), EtOH (5 mL) was added to the above reaction mixture and stirred for 10 min. The above mixture was poured into water, extracted with $CHCl_3$, washed with brine and water, dried over anhyd $MgSO_4$, evaporated to give **4** (126 mg, 94% yield), which was purified on a column of silica gel with hexane-EtOAc (3:1 v/v). Mp 65.2–67.3 °C (hexane-EtOAc); R_f = 0.21 (hexane-EtOAc = 3:1 v/v); $[\alpha]_D^{26} +27.0^\circ$ (*c* 3.00, $CHCl_3$); IR (KBr, disk): ν 3474 cm^{-1} (OH), 1721 cm^{-1} (C=O); 1H NMR (600 MHz): δ 7.38–7.30 (5H, m, PhH), 5.42–5.37 (2H, m, H-3'), 4.79, 4.59 (2H, each d, $J_{A,B}$ = 12.5 Hz, CH_2Ph), 4.72 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 4.50 (1H, dd, $J_{6a,5}$ = 5.0 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.27 (1H, dd, $J_{6b,5}$ = 2.2 Hz, $J_{6b,6a}$ = 12.2 Hz, H-6b), 3.91 (1H, m, H-2), 3.81 (1H, br dd, $J_{4,OH}$ = 6.4 Hz, $J_{4,5}$ = 9.3 Hz, H-4), 3.63 (1H, ddd, $J_{5,4}$ = 9.3 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 3.40 (3H, s, OCH_3), 2.71 (1H, d, $J_{OH,4}$ = 6.4 Hz, OH), 1.22 (9H, s, $C(CH_3)_3$). ^{13}C NMR (150 MHz): δ 179.4, 142.6, 137.8, 130.1, 128.6, 128.5, 128.4, 128.2, 127.9, 127.7, 105.9, 98.5, 76.6, 72.7, 72.5, 72.2, 68.2, 63.8, 63.7, 55.2, 38.9, 27.2. Anal. Calcd for $C_{20}H_{28}O_6$ (364.43): C, 65.91; H, 7.74%. Found: C, 65.83; H, 7.88%.

Methyl 2-O-Benzyl-3-deoxy-4-O-methoxymethyl-3-methylene-6-O-pivaloyl- α -D-ribo-hexopyranoside (5). To a solution of compound **4** (267.4 mg, 0.74 mmol) in dry CH_2Cl_2 (4 mL), dimethoxymethane (3.27 mL, 37 mmol), Molecular Sieves 4A (945 mg), and P_2O_5 (650 mg, 4.52 mmol) were added and stirred at rt for 5 h. After the disappearance of starting compound **4** on TLC with hexane-EtOAc (1:1 v/v), the reaction mixture was poured into saturated $NaHCO_3$ solution, extracted with $CHCl_3$, washed with brine and water, dried over anhyd $MgSO_4$, evaporated to give **5** (245.3 mg, 82% yield), which was purified on a column of silica gel with hexane-EtOAc (4:1 v/v). Colorless syrup; R_f = 0.47 (hexane-EtOAc = 2:1 v/v); $[\alpha]_D^{25} +123.0^\circ$ (*c* 1.80, $CHCl_3$); IR (KBr, neat): ν 1732 cm^{-1} (C=O); 1H NMR (600 MHz): δ 7.38–7.30 (5H, m, PhH), 5.39–5.27 (2H, m, H-3'), 4.79, 4.57 (2H, each

d, $J_{A,B}$ = 12.4 Hz, CH_2Ph), 4.74, 4.66 (2H, each d, $J_{A,B}$ = 6.9 Hz, OCH_2OCH_3), 4.71 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 4.40 (1H, dd, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.18 (1H, dd, $J_{6b,5}$ = 5.7 Hz, $J_{6b,6a}$ = 11.9 Hz, H-6b), 3.98 (1H, br d, $J_{4,5}$ = 9.8 Hz, H-4), 3.91 (1H, m, H-2), 3.75 (1H, ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 5.7 Hz, H-5), 3.42, 3.39 (6H, each s, $2 \times OCH_3$), 1.21 (9H, s, $C(CH_3)_3$). ^{13}C NMR (150 MHz): δ 178.2, 140.7, 137.9, 128.5, 127.9, 127.7, 106.4, 98.2, 76.9, 73.4, 72.1, 71.2, 63.3, 56.5, 55.1, 38.9, 27.2. Anal. Calcd for $C_{22}H_{32}O_7$ (408.49): C, 64.69; H, 7.90%. Found: C, 64.74; H, 8.16%.

Methyl 2-O-Benzyl-4-O-methoxymethyl-6-O-pivaloyl- α -D-glucopyranoside-3-spiro-2'-oxirane (6). To a solution of **5** (53 mg, 0.13 mmol) in dry $(CH_2Cl)_2$ (1 mL), *m*-chloroperbenzoic acid (44.9 mg, 0.26 mmol) was added and stirred at 55 °C under argon for 3 h. After the disappearance of starting compound **5** on TLC with hexane-MeOH (5:1 v/v), the reaction mixture was poured into saturated aq $NaHCO_3$ solution, extracted with $CHCl_3$, washed with brine and water, dried over anhyd $MgSO_4$, evaporated to give epoxide **6** (41 mg, 75% yield), which was purified on a column of silica gel with hexane-EtOAc (4:1 v/v). Colorless syrup; R_f = 0.48 (hexane-EtOAc = 2:1 v/v); $[\alpha]_D^{26} +91.5^\circ$ (*c* 1.26, $CHCl_3$); IR (KBr, neat): ν 1732 cm^{-1} (C=O); 1H NMR (600 MHz): δ 7.37–7.28 (5H, m, PhH), 4.78, 4.52 (2H, each d, $J_{A,B}$ = 12.2 Hz, CH_2Ph), 4.72, 4.51 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.69 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1), 4.41 (1H, dd, $J_{6a,5}$ = 2.1 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.17 (1H, dd, $J_{6b,6a}$ = 11.9 Hz, $J_{6b,5}$ = 5.7 Hz, H-6b), 3.88 (1H, ddd, $J_{5,4}$ = 10.1 Hz, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 5.7 Hz, H-5), 3.79 (1H, d, $J_{4,5}$ = 10.1 Hz, H-4), 3.70 (1H, m, H-2), 3.39, 3.36 (6H, each s, $2 \times OCH_3$), 3.13, 3.06 (2H, each d, $J_{a,b}$ = 5.8 Hz, H-3'), 1.21 (9H, s, $C(CH_3)_3$). ^{13}C NMR (150 MHz): δ 178.2, 137.8, 129.8, 128.4, 127.9, 98.9, 97.6, 73.9, 73.5, 70.4, 69.3, 63.1, 60.6, 56.4, 55.2, 45.4, 38.9, 27.2. Anal. Calcd for $C_{22}H_{32}O_8$ (424.48): C, 62.25; H, 7.60%. Found: C, 62.23; H, 7.57%.

Methyl 2-O-Benzyl-3-C-hydroxymethyl-4-O-methoxymethyl- α -D-glucopyranoside (7). To a solution of epoxide **6** (41 mg, 97.0 μ mol) in dry THF (1 mL), tetra-*n*-butylammonium hydroxide (40 wt % in aqueous solution, 315 μ L, 0.485 mmol) was added and refluxed for 30 h. After the disappearance of starting compound **6** on TLC with hexane-EtOAc (1:4 v/v), the reaction mixture was poured into saturated aq NH_4Cl solution, extracted with $CHCl_3$, washed with brine and water, dried over anhyd $MgSO_4$, evaporated to give diol **7** (29 mg, 83% yield), which was purified on a column of silica gel with hexane-EtOAc (1:4 v/v). Colorless syrup; R_f = 0.31 (hexane-EtOAc = 1:4 v/v); $[\alpha]_D^{26} +75.7^\circ$ (*c* 0.75, $CHCl_3$); IR (KBr, neat): ν 3474 cm^{-1} (OH); 1H NMR (600 MHz): δ 7.36–7.30 (5H, m, PhH), 4.90, 4.69 (2H, each d, $J_{A,B}$ = 6.4 Hz, OCH_2OCH_3), 4.78, 4.68 (2H, each d, $J_{A,B}$ = 11.9 Hz, CH_2Ph), 4.62 (1H, d, $J_{1,2}$ = 4.1 Hz, H-1), 4.18 (1H, dd, $J_{6a,OH}$ = 6.7 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 3.95 (1H, dd, $J_{6b,6a}$ = 11.9 Hz, $J_{6b,OH}$ = 7.2 Hz, H-6b), 3.91 (1H, d, $J_{OH,3'}$ = 2.1 Hz, OH), 3.80, 3.77 (2H, each ddd, $J_{3',OH}$ = 2.1 Hz, $J_{3',OH}$ = 6.5 Hz, $J_{a,b}$ = 12.2 Hz, H-3'), 3.67 (1H, d, $J_{4,5}$ = 10.4 Hz, H-4), 3.66 (1H, d, $J_{5,4}$ = 10.4 Hz, H-5), 3.59 (1H, d, $J_{2,1}$ = 4.1 Hz, H-2), 3.45, 3.36 (6H, each s, OCH_3), 2.95 (1H, dd, $J_{OH,6a}$ = 7.2 Hz, $J_{OH,6b}$ = 7.2 Hz, CH_2OH), 2.45 (1H, dd, $J_{OH,3'a}$ = 6.7 Hz, $J_{OH,3'b}$ = 6.7 Hz, OH). ^{13}C NMR (150 MHz): δ 137.6, 128.5, 128.1, 128.0, 99.2, 98.3, 81.5, 79.4, 75.2, 74.1, 69.1, 62.9, 61.7, 56.5, 55.5. Anal. Calcd for $C_{17}H_{26}O_8$ (358.38): C, 56.97; H, 7.31%. Found: C, 56.76; H, 7.37%.

Methyl 2-O-Benzyl-3-C-hydroxymethyl-4-O-methoxymethyl- α -D-glucopyranoside-3-spiro-4'-(2',2'-dimethyldioxolane) (8).

To a solution of diol **7** (2.52 g, 7.07 mmol) in CH_2Cl_2 (75 mL), acetone dimethylacetal (4.3 mL, 35.4 mmol) and *p*-toluenesulfonic acid monohydrate (54 mg, 0.28 mmol) were added and stirred for 20 min. After the disappearance of starting compound **7** on TLC with hexane–EtOAc (1:4 v/v), triethylamine (1 mL) was added to the reaction mixture and stirred 5 min. The above mixture was poured into saturated aq NaHCO_3 solution, extracted with CHCl_3 , washed with brine and water, dried over anhyd MgSO_4 , and evaporated to give acetone **8** (2.10 g, 75% yield), which was purified on a column of silica gel with hexane–EtOAc (1:1 v/v). Colorless syrup; R_f = 0.25 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{26} +94.7^\circ$ (*c* 0.82, CHCl_3); IR (KBr, neat): ν 3480 cm^{-1} (OH); ^1H NMR (500 MHz): δ 7.37–7.28 (5H, m, PhH), 4.90, 4.71 (2H, each d, $J_{\text{A,B}}$ = 6.5 Hz, OCH_2OCH_3), 4.83, 4.56 (2H, each d, $J_{\text{A,B}}$ = 12.4 Hz, CH_2Ph), 4.41 (1H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.35, 4.01 (2H, each d, $J_{\text{a,b}}$ = 9.3 Hz, H-3'), 3.92 (1H, br ddd, $J_{6\text{a},5}$ = 2.2 Hz, $J_{6\text{a},\text{OH}}$ = 5.2 Hz, $J_{6,6'}$ = 12.7 Hz, H-6a), 3.70 (1H, br ddd, $J_{6\text{b},6\text{a}}$ = 12.7 Hz, $J_{6\text{b},5}$ = 2.2 Hz, $J_{6\text{b},\text{OH}}$ = 8.9 Hz, H-6b), 3.57 (1H, d, $J_{4,5}$ = 10.1 Hz, H-4), 3.53 (1H, d, $J_{2,1}$ = 3.4 Hz, H-2), 3.45 (3H, s, OCH_3), 3.43 (1H, ddd, $J_{5,4}$ = 10.1 Hz, $J_{5,6\text{a}}$ = 2.2 Hz, $J_{5,6\text{b}}$ = 2.2 Hz, H-5), 3.28 (3H, s, OCH_3), 3.08 (1H, br dd, $J_{\text{OH},6\text{a}}$ = 5.2 Hz, $J_{\text{OH},6\text{b}}$ = 8.9 Hz, OH), 1.48, 1.44 (6H, each s, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz): δ 137.9, 128.5, 128.5, 128.2, 128.0, 109.2, 100.2, 98.9, 85.8, 78.2, 78.1, 74.4, 70.4, 64.5, 61.5, 56.5, 55.3, 26.9, 25.9. Anal. Calcd for $\text{C}_{20}\text{H}_{30}\text{O}_8$ (398.45): C, 60.29; H, 7.59%. Found: C, 59.97; H, 7.52%.

Methyl 6-O-Acetyl-2-O-benzyl-4-O-methoxymethyl- α -D-xyllo-hex-5-enopyranoside-3-spiro-4'-(2',2'-dimethyldioxolane) (9). To a stirred solution of trifluoroacetic anhydride (200 μL , 1.36 mmol) in dry CH_2Cl_2 (30 mL), dimethyl sulfoxide (170 μL , 2.4 mmol) was added at -78°C under argon. After 15 min, a solution of alcohol **8** (94.8 mg, 0.24 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise to the reaction mixture at -78°C . After 30 min, triethylamine (400 μL , 2.88 mmol) was added dropwise to the above mixture, and stirred for 5 min. After the disappearance of starting compound **8** on TLC with toluene–acetone (8:1 v/v), the reaction mixture was warm to rt, poured into saturated aq NaHCO_3 solution, extracted with CHCl_3 , washed with brine and water, dried over anhyd MgSO_4 , and evaporated to give unstable aldehyde. Following, to a solution of aldehyde in dry CH_3CN (5 mL), potassium carbonate (199 mg, 1.44 mmol) and acetic anhydrous (227 μL , 2.4 mmol) were added and refluxed for 6 h. After the disappearance of starting aldehyde on TLC with hexane–EtOAc (1:1 v/v), the reaction mixture was poured into saturated aq NH_4Cl solution, extracted with EtOAc, washed with brine and water, dried over anhyd MgSO_4 , and evaporated to give enol acetate **9** (75.2 mg, 2 steps 72% yield), which was purified on a column of silica gel with hexane–EtOAc (1:1 v/v). Mp 50.0–51.0 $^\circ\text{C}$ (colorless plates, hexane–EtOAc); R_f = 0.47 (hexane–EtOAc = 2:1 v/v); $[\alpha]_D^{26} -10.6^\circ$ (*c* 0.97, CHCl_3); IR (KBr, disk): ν 1755 cm^{-1} (C=O), 1639 cm^{-1} (C=C); ^1H NMR (600 MHz): δ 7.39–7.31 (5H, m, PhH), 7.21 (1H, d, $J_{6,4}$ = 1.9 Hz, H-6), 4.88, 4.61 (2H, each d, $J_{\text{A,B}}$ = 12.4 Hz, CH_2Ph), 4.87, 4.75 (2H, each d, $J_{\text{A,B}}$ = 6.5 Hz, OCH_2OCH_3), 4.53 (1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.21, 3.98 (2H, each d, $J_{\text{a,b}}$ = 9.3 Hz, H-3'), 4.03 (1H, d, $J_{4,6}$ = 1.9 Hz, H-4), 3.64 (1H, d, $J_{2,1}$ = 3.4 Hz, H-2), 3.47, 3.41 (6H, each s, $2 \times \text{OCH}_3$), 2.14 (3H, s, COCH_3), 1.48, 1.44 (6H, each s, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz): δ 167.2, 137.8, 135.0, 128.5, 128.2, 128.1, 123.7, 109.9, 100.4, 98.2, 85.4, 77.4, 76.2, 74.7, 64.4, 56.9, 56.4, 26.7, 25.7, 20.5. Anal. Calcd for $\text{C}_{22}\text{H}_{30}\text{O}_9$ (438.47): C, 60.26; H, 6.90%. Found: C, 60.50; H, 6.87%.

Aldehyde (Methyl (5R)-2-O-Benzyl-4-O-methoxymethyl- α -

D-gluco-hexodialdopyranoside-3-spiro-4'-(2',2'-dimethyldioxolane)): Colorless syrup; R_f = 0.25 (toluene–acetone = 8:1 v/v); ^1H NMR (270 MHz): δ 9.63 (1H, d, $J_{\text{CHO},5}$ = 2.3 Hz), 8.28–7.23 (5H, m, PhH), 4.89, 4.67 (2H, each d, $J_{\text{A,B}}$ = 12.2 Hz, CH_2Ph), 4.85, 4.56 (2H, each d, $J_{\text{A,B}}$ = 6.6 Hz, OCH_2OCH_3), 4.46 (1H, $J_{1,2}$ = 3.3 Hz, H-1), 4.33, 4.09 (2H, each d, $J_{\text{a,b}}$ = 9.5 Hz, H-3'), 3.87 (1H, dd, $J_{5,\text{CHO}}$ = 2.3 Hz, $J_{5,4}$ = 10.2 Hz, H-5), 3.65 (1H, d, $J_{4,5}$ = 10.2 Hz, H-4), 3.53 (1H, d, $J_{2,1}$ = 3.3 Hz, H-2), 3.30, 3.29 (6H, each s, $2 \times \text{OCH}_3$), 1.47, 1.44 (6H, each s, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz): δ 197.0, 137.7, 128.6, 128.2, 128.2, 109.6, 99.7, 99.0, 85.4, 78.0, 77.7, 74.6, 74.6, 74.2, 64.5, 56.3, 55.9, 26.8, 25.8. ESI-TOF-MS Calcd for $\text{C}_{20}\text{H}_{29}\text{O}_8$ m/z [$\text{M} + \text{H}$] $^+$: 397.1862. Found: 397.1865.

2D-(2,3,4,6/5(OH))-2-O-Acetyl-4-O-benzyl-5-C-hydroxymethyl-5,5'-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone (10a), 2D-(2,4,6/3,5(OH))-2-O-Acetyl-4-O-benzyl-5-C-hydroxymethyl-5,5'-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone (10b), 2L-(3,4,6/2,5(OH))-2-O-Acetyl-4-O-benzyl-5-C-hydroxymethyl-5,5'-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone (10c), and 2L-(4,6/2,3,5(OH))-2-O-Acetyl-4-O-benzyl-5-C-hydroxymethyl-5,5'-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone (10d). To a solution of enol acetate **9** (218 mg, 0.497 mmol) in a mixture of acetone– H_2O –AcOH (500:200:7 v/v/v, 6 mL), mercury(II) acetate (205 mg, 0.464 mmol) was added and stirred at 70°C for 1 h. After the disappearance of starting compound **9** on TLC with hexane–EtOAc (1:1 v/v), the reaction mixture was cooled to rt, saturated aq NaCl solution (3 mL) was added to the above mixture, and stirred for 5 min. After the end of reaction as determined by TLC with hexane–EtOAc (1:1 v/v), the mixture was extracted with CHCl_3 , washed with brine and water, and evaporated to give a residue. The remaining residue was purified on a column of silica gel with hexane–EtOAc (4:1 v/v) to give cyclitol **10a** (122 mg, 58% yield) and its diastereomers **10b** (18 mg, 23% yield) and **10c** (9.2 mg, 9% yield), respectively.

10a: colorless syrup; R_f = 0.34 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{25} -8.74^\circ$ (*c* 2.31, CHCl_3); IR (KBr neat): ν 3543 cm^{-1} (OH), 1748, 1732 cm^{-1} (C=O); ^1H NMR (600 MHz): δ 7.41–7.35 (5H, m, PhH), 5.21 (1H, dd, $J_{2,6}$ = 1.0 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.92, 4.73 (2H, each d, $J_{\text{A,B}}$ = 11.9 Hz, CH_2Ph), 4.78, 4.75 (2H, each d, $J_{\text{A,B}}$ = 6.7 Hz, OCH_2OCH_3), 4.31 (1H, d, $J_{6,2}$ = 1.0 Hz, H-6), 4.26, 3.90 (2H, each d, $J_{\text{A,B}}$ = 9.5 Hz, H-5'), 4.21 (1H, ddd, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 2.2 Hz, $J_{3,\text{OH}}$ = 1.5 Hz, H-3), 4.02 (1H, d, $J_{4,3}$ = 2.2 Hz, H-4), 3.43 (3H, s, OCH_3), 2.29 (1H, d, $J_{\text{OH},3}$ = 1.5 Hz, OH), 2.20 (3H, s, COCH_3), 1.52, 1.46 (6H, each s, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz): δ 197.0, 169.4, 137.1, 128.7, 128.4, 128.1, 110.2, 96.9, 87.2, 81.1, 77.8, 74.7, 74.5, 69.4, 64.6, 56.5, 26.6, 26.0, 20.5. Anal. Calcd for $\text{C}_{21}\text{H}_{28}\text{O}_9$ (424.44): C, 59.43; H, 6.65%. Found: C, 59.34; H, 7.05%. ESI-TOF-MS Calcd for $\text{C}_{21}\text{H}_{28}\text{NaO}_9$ m/z [$\text{M} + \text{Na}$] $^+$: 447.1631. Found: 447.1596.

10b: colorless syrup; R_f = 0.48 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{25} -14.4^\circ$ (*c* 1.01, CHCl_3); IR (KBr neat): ν 3468 cm^{-1} (OH), 1755, 1748 cm^{-1} (C=O); ^1H NMR (600 MHz): δ 7.40–7.33 (5H, m, PhH), 5.23 (1H, dd, $J_{2,6}$ = 1.0 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 5.03, 4.80 (2H, each d, $J_{\text{A,B}}$ = 11.7 Hz, OCH_2OCH_3), 4.77 (2H, s, CH_2Ph), 4.47 (1H, d, $J_{6,2}$ = 1.0 Hz, H-6), 3.98, 3.81 (2H, each d, $J_{\text{a,b}}$ = 8.4 Hz, H-5'), 3.92 (1H, d, $J_{4,3}$ = 9.5 Hz, H-4), 3.48 (1H, d, $J_{3,4}$ = 9.5 Hz, H-3), 3.43 (3H, s, OCH_3), 2.50 (1H, br s, OH), 2.18 (3H, s, COCH_3), 1.52, 1.48 (6H, each s, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (150 MHz): δ 197.0, 169.7, 137.6, 128.9, 128.4, 127.9, 111.7, 96.7, 84.6, 81.6, 80.2, 77.3, 76.5, 71.1, 64.3, 56.5, 26.4, 26.3, 20.5. ESI-

TOF-MS Calcd for $C_{21}H_{28}NaO_9$ m/z $[M + Na]^+$: 447.16310. Found: 447.1602.

10c: colorless syrup; R_f = 0.23 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{25}$ +8.9° (c 0.1, $CHCl_3$); IR (KBr neat): ν 3495 cm^{-1} (OH), 1744, 1742 cm^{-1} (C=O); 1H NMR (600 MHz): δ 7.40–7.34 (5H, m, PhH), 6.00 (1H, d, $J_{2,3}$ = 3.3 Hz, H-2), 4.83, 4.64 (2H, each d, $J_{A,B}$ = 11.9 Hz, CH_2 Ph), 4.69, 4.67 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.43 (1H, ddd, $J_{3,4}$ = 3.6 Hz, $J_{3,OH}$ = 9.6 Hz, $J_{3,2}$ = 3.3 Hz, H-3), 4.22, 3.99 (2H, each d, $J_{a,b}$ = 9.6 Hz, H-5'), 3.98 (1H, d, $J_{6,4}$ = 1.7 Hz, H-6), 3.50 (1H, dd, $J_{4,3}$ = 3.6 Hz, $J_{4,6}$ = 1.7 Hz, H-4), 3.39 (3H, s, OCH_3), 3.13 (1H, d, $J_{OH,3}$ = 9.6 Hz, OH), 2.23 (3H, s, $COCH_3$), 1.41, 1.32 (6H, each s, $C(CH_3)_2$). ^{13}C NMR (150 MHz): δ 200.2, 170.0, 136.9, 128.6, 128.3, 128.0, 112.5, 96.5, 85.2, 82.6, 79.8, 74.9, 73.4, 73.0, 69.2, 56.5, 26.6, 26.2, 20.7. ESI-TOF-MS Calcd for $C_{21}H_{28}NaO_9$ m/z $[M + Na]^+$: 447.1631. Found: 447.1662.

10d: colorless syrup; R_f = 0.6–0.2 tailing (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{26}$ +10.3° (c 0.1, $CHCl_3$); IR (KBr neat): ν 3468 cm^{-1} (OH), 1745, 1741 cm^{-1} (C=O); 1H NMR (600 MHz): δ 7.43–7.27 (5H, m, PhH), 5.37 (1H, dd, $J_{2,3}$ = 4.1 Hz, $J_{2,4}$ = 3.3 Hz, H-2), 5.14 (1H, dd, $J_{3,4}$ = 4.3 Hz, $J_{3,OH}$ = 7.0 Hz, H-3), 4.89, 4.68 (2H, each d, $J_{A,B}$ = 12.0 Hz, CH_2 Ph), 4.67, 4.65 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.26, 3.85 (2H, each d, $J_{a,b}$ = 9.6 Hz, H-5'), 3.95 (1H, d, $J_{6,4}$ = 1.2 Hz, H-6), 3.94 (1H, dd, $J_{4,6}$ = 1.2 Hz, $J_{4,3}$ = 4.3 Hz, H-4), 3.37 (3H, s, OCH_3), 3.20 (1H, d, $J_{OH,3}$ = 7.0 Hz, OH), 2.00 (3H, s, $COCH_3$), 1.36, 1.13 (6H, each s, $C(CH_3)_2$). ^{13}C NMR (150 MHz): δ 205.6, 170.5, 137.0, 128.5, 128.4, 128.2, 128.0, 111.5, 97.0, 84.7, 82.5, 74.9, 73.3, 69.6, 69.0, 56.5, 26.7, 26.1, 20.9. ESI-TOF-MS Calcd for $C_{21}H_{28}NaO_9$ m/z $[M + Na]^+$: 447.1631. Found: 447.1643.

2d-(2,3,4,6/5(OH))-2-O-Acetyl-5-C-hydroxymethyl-3,4,5,5'-di-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone (11). A solution of cyclitol **10a** (146 mg, 0.344 mmol) in EtOH (7 mL) was hydrogenated in the presence of a catalytic amount of 20% Pd(OH)₂-C (116 mg) under H₂ gas at rt for 2 h. After the disappearance of starting compound **10a** on TLC with toluene–acetone (1:1 v/v), catalyst was filtered off, and evaporated to quantitatively give diol. To a solution of this diol (10.6 mg, 31.7 μ mol) in a mixture of acetone– CH_2Cl_2 (1:1 v/v) (0.4 mL), 2,2-dimethoxypropane (40 μ L, 0.317 mmol) and *p*-toluenesulfonic acid monohydrate (5.5 mg, 3.17 μ mol) were added and stirred at rt for 1 h. After the disappearance of starting diol on TLC with toluene–acetone (2:1 v/v), the reaction mixture was quenched with saturated aq NaHCO₃ solution, washed with brine and water, dried over anhyd MgSO₄, and evaporated to give diacetone **11** (7.8 mg, 66% yield), which was purified on a column of silica gel with hexane–EtOAc (2:1 v/v). Mp 145.0–146.0 °C (colorless prism, hexane–EtOAc); R_f = 0.41 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{25}$ –152.4° (c 1.03, $CHCl_3$); IR (KBr disk): ν 1759, 1741 cm^{-1} (C=O); 1H NMR (600 MHz): δ 5.70 (1H, d, $J_{2,3}$ = 4.5 Hz, H-2), 5.03, 4.72 (2H, each d, $J_{a,b}$ = 6.9 Hz, OCH_2OCH_3), 4.84 (1H, dd, $J_{3,2}$ = 4.5 Hz, $J_{3,4}$ = 6.8 Hz, H-3), 4.43 (1H, d, $J_{4,3}$ = 6.8 Hz, H-4), 4.35, 4.24 (2H, each d, $J_{A,B}$ = 9.8 Hz, H-5'), 4.18 (1H, s, H-6), 3.46 (3H, s, OCH_3), 2.24 (3H, s, $COCH_3$), 1.48, 1.45, 1.39, 1.35 (12H, each s, $2 \times C(CH_3)_2$). ^{13}C NMR (150 MHz): δ 200.6, 170.0, 111.7, 111.5, 97.9, 82.1, 80.2, 77.4, 75.9, 73.8, 69.3, 56.6, 27.1, 25.9, 25.6, 24.4, 20.6. Anal. Calcd for $C_{17}H_{26}O_9$ (374.38): C, 54.54; H, 7.00%. Found: C, 54.41; H, 7.24%.

Diol (2d-(2,3,4,6/5(OH))-2-O-Acetyl-5-C-hydroxymethyl-5,5'-O-isopropylidene-6-O-methoxymethyl-2,3,4,5,6-pentahydroxycyclohexanone): Mp 66.5–67.3 °C (hexane–EtOH);

R_f = 0.27 (toluene–acetone = 1:1 v/v); $[\alpha]_D^{25}$ +19.4° (c 3.40, $CHCl_3$); IR (KBr, disk): ν 3458 cm^{-1} (OH), 1730, 1714 cm^{-1} (C=O); 1H NMR (600 MHz): δ 5.33 (1H, d, $J_{2,3}$ = 3.4 Hz, H-2), 4.76 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.46 (1H, br dd, $J_{3,OH}$ = 2.8 Hz, $J_{3,2}$ = 2.8 Hz, H-3), 4.41 (1H, s, H-6), 4.30, 3.96 (2H, each d, $J_{a,b}$ = 9.8 Hz, H-5'), 4.22 (1H, br s, H-4), 3.32 (3H, s, OCH_3), 2.84 (1H, br d, $J_{OH,4}$ = 3.4 Hz, OH), 2.64 (1H, br s, OH), 2.23 (3H, s, $COCH_3$), 1.49 (6H, s, $C(CH_3)_2$). ^{13}C NMR (150 MHz): δ 197.6, 169.7, 110.1, 97.0, 87.5, 80.0, 75.3, 71.8, 71.2, 64.3, 56.8, 26.8, 26.3, 20.7. Anal. Calcd for $C_{14}H_{22}O_9$ (334.32): C, 50.30; H, 6.63%. Found: C, 49.99; H, 6.92%.

1d-(1,2,3,5/4(OH))-1-O-tert-Butyldimethylsilyl-4-C-hydroxymethyl-2,3:4,4'-di-O-isopropylidene-5-O-methoxymethyl-6-C-methylene-1,2,3,4,5-cyclohexanepentol (12). To a solution of diacetone **11** (60 mg, 0.16 mmol) in dry Et₂O (10 mL), trimethylsilylmethylmagnesium chloride (1.0 M in Et₂O solution, 2.88 mL, 2.88 mmol) was added dropwise at 0 °C, and stirred for 20 h. After the disappearance of starting compound **11** on TLC with hexane–EtOAc (1:1 v/v), the reaction mixture was poured into saturated aq NH₄Cl solution, extracted with EtOAc, washed with brine and water, dried over anhyd MgSO₄, and evaporated to give olefin (30 mg, 56% yield), which was purified on a column of silica gel with hexane–EtOAc (3:2 v/v). To a solution of resulting olefin (30 mg, 80 μ mol) in dry CH_2Cl_2 (4 mL) *tert*-butyldimethylsilyl chloride (78 mg, 0.51 mmol) and imidazole (52 mg, 0.77 mmol) were added and stirred for 22 h. After the disappearance of starting olefin on TLC with hexane–EtOAc (2:1 v/v), the reaction mixture was poured into saturated aq NaHCO₃ solution, extracted with $CHCl_3$, washed with brine and water, and evaporated to give silyl ether **12** (28.8 mg, 88% yield), which was purified on a column of silica gel with hexane–EtOAc (4:1 v/v). Colorless syrup; R_f = 0.36 (hexane–EtOAc = 4:1 v/v); $[\alpha]_D^{25}$ –48.4° (c 3.38, $CHCl_3$); IR: Characteristic absorption was not observed; 1H NMR (600 MHz): δ 5.43, 5.31 (2H, each m, H-6'), 4.79, 4.75 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.53 (1H, m, H-1), 4.41 (1H, dd, $J_{2,1}$ = 4.5 Hz, $J_{2,3}$ = 6.9 Hz, H-2), 4.21 (1H, d, $J_{3,2}$ = 6.9 Hz, H-3), 4.15 (2H, each s, H-4'), 4.14 (1H, br s, H-5), 3.43 (3H, s, OCH_3), 1.47, 1.43, 1.40, 1.33 (12H, each s, $2 \times C(CH_3)_2$), 0.94 (9H, s, $SiC(CH_3)_3$), 0.14, 0.12 (6H, each s, $Si(CH_3)_2$). ^{13}C NMR (150 MHz): δ 143.1, 112.1, 110.2, 109.8, 96.5, 83.3, 79.2, 77.6, 69.5, 67.7, 56.1, 26.9, 26.3, 26.0, 25.7, 24.8, 18.6, 13.7, –4.6, –4.9. Anal. Calcd for $C_{22}H_{40}O_7Si$ (444.63): C, 59.43; H, 9.07%. Found: C, 59.62; H, 8.85%.

Olefin (1d-(1,2,3,5/4(OH))-4-C-Hydroxymethyl-2,3:4,4'-di-O-isopropylidene-5-O-methoxymethyl-6-C-methylene-1,2,3,4,5-cyclohexanepentol): Mp 55.5–56.5 °C (colorless needles, hexane–EtOAc); R_f = 0.40 (hexane–EtOAc = 1:1 v/v); $[\alpha]_D^{25}$ –104.3° (c 0.45, $CHCl_3$); IR (KBr disk): ν 3487 cm^{-1} (OH), 1659 cm^{-1} (C=C); 1H NMR (600 MHz): δ 5.37, 5.33 (2H, each m, H-6'), 4.79, 4.75 (2H, each d, $J_{A,B}$ = 6.7 Hz, OCH_2OCH_3), 4.48 (1H, dd, $J_{4,5}$ = 4.8 Hz, $J_{4,3}$ = 6.7 Hz, H-2), 4.36 (1H, br ddd, $J_{5,4}$ = 4.8 Hz, $J_{5,1}$ = 1.9 Hz, $J_{5,OH}$ = 9.8 Hz, H-5), 4.28 (1H, dd, $J_{3,4}$ = 6.7 Hz, $J_{3,1}$ = 0.7 Hz, H-3), 4.26, 4.16 (2H, each d, $J_{A,B}$ = 9.5 Hz, H-4'), 4.24 (1H, br d, $J_{1,3}$ = 0.7 Hz, H-1), 3.45 (3H, s, OCH_3), 2.43 (1H, d, $J_{OH,5}$ = 9.8 Hz, OH), 1.44, 1.43, 1.41, 1.35 (12H, each s, $2 \times C(CH_3)_2$). ^{13}C NMR (150 MHz): δ 143.6, 112.3, 110.5, 110.1, 97.1, 81.8, 79.5, 78.3, 76.1, 68.6, 68.3, 56.4, 27.0, 26.2, 25.6, 25.0. Anal. Calcd for $C_{16}H_{26}O_7$ (330.37): C, 58.17; H, 7.93%. Found: C, 57.90; H, 8.20%.

1d-(1,2,3,5,6/4(OH))-4,6-Di-C-hydroxymethyl-2,3:4,4'-di-O-isopropylidene-5-O-methoxymethyl-1,2,3,4,5-cyclohexanepentol (13). To a solution of olefin **12** (215 mg, 0.484 mmol) in dry THF (15 mL), Borane–THF complex (1.0 M in THF solution, 1.45 mL,

1.45 mmol) was added at 0 °C, and stirred at rt. After 1 h, 10 wt % aq NaOH solution (9 mL) was added to the stirred mixture at 0 °C. After 5 min, 30 wt % aq H₂O₂ solution (9 mL) was added and stirred at 0 °C for 1 h. After monitoring of the reaction on TLC with hexane–EtOAc (2:1 v/v), the reaction mixture was poured into saturated aq NH₄Cl solution, extracted with EtOAc, washed with sodium thiosulfate solution, brine, and water, dried over anhyd MgSO₄, and evaporated to give residue. To a solution of the remaining residue in tetrahydrofuran (5 mL), tetra-*n*-butylammonium fluoride (1.0 M in THF solution, 0.58 mL, 0.58 mmol) was added and stirred for 1 h at rt. After the disappearance of starting compound **12** on TLC with EtOAc, the reaction mixture was evaporated to give a residue. The remaining residue was purified on a column of silica gel with EtOAc to give diol **13** (126 mg, 74% yield 2 steps). Colorless syrup; *R*_f = 0.55 (EtOAc); $[\alpha]_D^{25} +6.59^\circ$ (*c* 1.8, CHCl₃); IR (KBr, neat): ν 3464 cm⁻¹ (OH); ¹H NMR (600 MHz): δ 4.77, 4.72 (2H, each d, *J*_{A,B} = 6.7 Hz, OCH₂OCH₃), 4.26 (2H, each d, *J*_{A,B} = 9.6 Hz, H-4'), 4.15 (1H, d, *J*_{2,1} = 4.8 Hz, H-2), 4.15 (1H, s, H-3), 4.01 (1H, dd, *J*_{6'a,6} = 9.1 Hz, *J*_{6'a,6'b} = 11.3 Hz, H-6'a), 3.93 (1H, d, *J*_{5,6} = 2.4 Hz, H-5), 3.85 (1H, dd, *J*_{6'b,6} = 6.2 Hz, *J*_{6'b,6'a} = 11.3 Hz, H-6'b), 3.83 (1H, br ddd, *J*_{1,2} = 4.8 Hz, *J*_{1,6} = 2.4 Hz, *J*_{1,OH} = 10.8 Hz, H-1), 3.46 (3H, s, OCH₃), 3.18 (1H, d, *J*_{OH,1} = 10.8 Hz, OH), 2.63 (1H, br s, OH), 2.10 (1H, dddd, *J*_{6,6'a} = 9.1 Hz, *J*_{6,5} = 2.4 Hz, *J*_{6,6'b} = 6.2 Hz, *J*_{6,1} = 2.4 Hz, H-6), 1.58, 1.41, 1.41, 1.40 (12H, each s, 2 × C(CH₃)₂). ¹³C NMR (150 MHz): δ 110.8, 109.7, 99.5, 80.6, 79.9, 78.3, 75.5, 69.3, 68.2, 61.3, 56.8, 39.7, 27.0, 26.6, 25.8, 25.7. Anal. Calcd for C₁₆H₂₈O₈ (348.39): C, 55.16; H, 8.10%. Found: C, 55.0; H, 8.20%.

1d-(1,2,3,5,6/4(OH))-6'-O-tert-Butyldiphenylsilyl-4,6-di-C-hydroxymethyl-2,3,4,4'-di-O-isopropylidene-5-O-methoxymethyl-1,2,3,4,5-cyclohexanepentol (14). To a solution of diol **13** (1.3 g, 3.73 mmol) in dry CH₂Cl₂ (45 mL) *tert*-butyldiphenylsilyl chloride (1.2 mL) and imidazole (1.0 mg, 14.7 mmol) were added and stirred for 10 min. After the disappearance of starting compound **13** on TLC with EtOAc, the reaction mixture was poured into saturated aq NaHCO₃ solution, extracted with CHCl₃, washed with brine and water, and evaporated to give silyl ether **14** (2.01 g, 92% yield), which was purified on a column of silica gel with hexane–EtOAc (4:1 v/v). Colorless syrup; *R*_f = 0.45 (hexane–EtOAc = 3:1 v/v); $[\alpha]_D^{26} -23.6^\circ$ (*c* 2.8, CHCl₃); IR (KBr, neat): ν 3508 cm⁻¹ (OH); ¹H NMR (600 MHz): δ 7.68–7.36 (10H, m, PhH), 4.71, 4.61 (2H, each d, *J*_{A,B} = 6.7 Hz, OCH₂OCH₃), 4.35, 4.30 (2H, each d, *J*_{A,b} = 9.6 Hz, H-4'), 4.14 (1H, d, *J*_{3,2} = 5.3 Hz, H-3), 4.11 (1H, dd, *J*_{2,3} = 5.3 Hz, *J*_{2,1} = 5.2 Hz, H-2), 4.06 (1H, br dd, *J*_{5,1} = 0.9 Hz, *J*_{5,6} = 2.2 Hz, H-5), 4.02 (1H, dd, *J*_{6'a,6} = 9.5 Hz, *J*_{6'a,6'b} = 10.3 Hz, H-6'a), 3.88 (1H, dd, *J*_{6'b,6'a} = 10.3 Hz, *J*_{6'b,6} = 6.2 Hz, H-6'b), 3.76 (1H, dddd, *J*_{1,2} = 5.2 Hz, *J*_{1,5} = 0.9 Hz, *J*_{1,6} = 2.1 Hz, *J*_{1,OH} = 11.3 Hz, H-1), 3.31 (3H, s, OCH₃), 3.30 (1H, d, *J*_{OH,1} = 11.3 Hz, OH), 2.09 (1H, dddd, *J*_{6,6'b} = 6.2 Hz, *J*_{6,6'a} = 9.5 Hz, *J*_{6,5} = 2.2 Hz, *J*_{6,1} = 2.1 Hz, H-6), 1.56, 1.45, 1.42, 1.40 (12H, each s, 2 × C(CH₃)₂), 1.05 (9H, s, C(CH₃)₃). ¹³C NMR (150 MHz): δ 135.6, 135.5, 133.5, 133.3, 129.7, 129.7, 127.7, 127.7, 110.5, 109.6, 98.9, 80.2, 80.0, 78.2, 75.5, 69.7, 68.1, 62.3, 56.2, 40.3, 26.8, 26.7, 25.8, 25.8, 19.2. NOE correlations: H-2–H-6; H-4'–H-3, H-5. Anal. Calcd for C₃₂H₄₆O₈Si (586.78): C, 65.50; H, 7.90%. Found: C, 65.17; H, 7.98%.

2d-(2,3,5,6/4(OH))-6'-O-tert-Butyldiphenylsilyl-4,6-di-C-hydroxymethyl-2,3,4,4'-di-O-isopropylidene-5-O-methoxymethyl-2,3,4,5-tetrahydroxycyclohexanone (15). To a solution of compound **14** (0.876 g, 1.49 mmol) in dry CH₂Cl₂ (20 mL), Dess–Martin periodinane (1.268 g, 2.99 mmol) was added and stirred for 15 min at rt. After the disappearance of starting compound **14** on

TLC with hexane–EtOAc (4:1 v/v), the reaction mixture was poured into saturated aq NaHCO₃ solution, extracted with CHCl₃, washed with brine and water, and evaporated to give ketone **15** (865 mg, 99% yield), which was purified on a column of silica gel with hexane–EtOAc (4:1 v/v). Colorless syrup; *R*_f = 0.4 (hexane–EtOAc = 4:1 v/v); $[\alpha]_D^{26} +17.0^\circ$ (*c* 0.83, CHCl₃); IR (KBr, neat): ν 1732 cm⁻¹ (C=O); ¹H NMR (600 MHz): δ 7.65–7.34 (10H, m, PhH), 4.62, 4.54 (2H, each d, *J*_{A,B} = 6.7 Hz, CH₂OCH₃), 4.44 (1H, dd, *J*_{3,2} = 6.0 Hz, *J*_{3,5} = 1.9 Hz, H-3), 4.43, 4.35 (2H, each d, *J*_{A,B} = 9.6 Hz, H-4'), 4.40 (1H, d, *J*_{2,3} = 6.0 Hz, H-2), 4.37 (1H, dd, *J*_{5,6} = 2.1 Hz, *J*_{5,3} = 1.9 Hz, H-5), 4.01 (1H, dd, *J*_{6'a,6} = 4.8 Hz, *J*_{6'a,6'b} = 11.0 Hz, H-6'a), 3.93 (1H, dd, *J*_{6'b,6'a} = 11.0 Hz, *J*_{6'b,6} = 10.3 Hz, H-6'b), 3.30 (3H, s, OCH₃), 3.28 (1H, ddd, *J*_{6,6'a} = 4.8 Hz, *J*_{6,6'b} = 10.3 Hz, *J*_{6,5} = 2.1 Hz, H-6), 1.51, 1.50, 1.36, 1.34 (12H, each s, 2 × C(CH₃)₂), 1.04 (9H, s, C(CH₃)₃). ¹³C NMR (150 MHz): δ 206.2, 135.5, 135.5, 134.8, 133.2, 129.8, 129.6, 127.7, 111.0, 110.8, 98.7, 82.8, 82.3, 79.2, 78.6, 69.8, 58.3, 55.7, 51.5, 26.9, 26.8, 26.8, 26.7, 26.5, 26.2, 19.2, 19.1. Anal. Calcd for C₃₂H₄₄O₈Si (584.77): C, 65.73; H, 7.58%. Found: C, 65.79; H, 7.33%.

(–)-Tetrodotoxin. White powder; *R*_f = 0.5 (1-butanol–AcOH–H₂O = 2:1:1 v/v/v), *R*_f = 0.6 (pyridine–EtOAc–AcOH–H₂O = 15:9:3:6 v/v/v/v); $[\alpha]_D^{28} -3.75^\circ$ (*c* 0.13, 3% AcOH); ¹H NMR (600 MHz, in 3% CD₃COOD/D₂O, referenced to CHD₂COOD (2.06 ppm)): δ 5.50 (d, 1H, *J* = 8.9 Hz, H-4), 4.30 (d, 1H, *J* = 2.1 Hz, H-8), 4.25 (br s, 1H, H-5), 4.09 (t, 1H, *J* = 2.1 Hz, H-7), 4.06, 4.01 (2 × d, 2H, *J* = 12.4 Hz, H-11), 3.96 (s, 1H, H-9), 2.35 (d, 1H, *J* = 9.6 Hz, H-4a). ESI-TOF-MS Calcd for C₁₁H₁₈N₃O₈ *m/z* [M + H]⁺: 320.1094. Found: 320.1085.

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Supporting Information

The copies of ¹H and ¹³C NMR (PDF). This material is available free of charge on the web at <http://www.csj.jp/journals/bcsj/>.

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