The Exciton Chirality Method in Vibrational Circular Dichroism

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Supporting Information

General Procedures:

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian Inova instrument at 25 C° in $CDCl_3$. Chemical shifts (δ) are reported in ppm relative to $CDCl_3$ (1H, d 7.26; ^{13}C, δ 77.00) or tetramethylsilane. Electron Ionization mass spectrometry was conducted by using a JEOL JMS-700TZ spectrometer. Electrospray ionization mass spectra were obtained either by a JEOL JMS-T100LP spectrometer or by a Thermo Scientific Exactive spectrometer. Optical rotations were measured on a JASCO P-1020 polarimeter at the sodium D-line using a 1-cm optical cell under ambient temperature, and reported as $[\alpha]_D$ (concentration in grams/100 mL solvent). VCD and IR spectra were measured on a BioTools Chiralir spectrometer equipped with a second photoelastic modulator. All spectra were recorded in $CDCl_3$ using a 100-μm CaF_2 cell at a resolution of 8 cm^{-1} at ambient temperature. All spectral data except those in Figure S5 were corrected by a solvent spectrum obtained under the same experimental condition, and presented as Δε and ε (both in M^{-1} cm^{-1}). Analytical TLC was performed on 0.2-mm silica-gel plates (Merch 60 F-254), and column chromatography was carried out with silica gel (Wakosil C-200, 64-210 μm). Chiral separation of **9** was performed by using two JASCO PU-2086 intelligent pumps equipped with a JASCO MX-2080-31 solvent mixing module with detection at 254 nm on a PU-2075 intelligent UV/Vis detector. Spectroscopic grade chloroform was purchased from Wako Pure Chemical Industries, Ltd., and $CDCl_3$ was purchased from Cambridge Isotope Laboratories. (*S*)-(−)-α-hydroxy-γ-butyrolactone,(*R*)-(+)-α-hydroxy-γ-butyrolactone,(*S*)-(−)-2-amino-4-butyro-lactone hydrobromide, *cis*-androsterone, diltiazem hydrochloride, penicillin G sodium salt were purchased from Wako Pure Chemical Industries, Ltd. *N*-Tetradecanoyl homoserine lactone was purchased from Cayman Chemical Company. Picrotoxinin and taxifolin were purchased from Extrasynthese. Chemicals were used without further purification, except the following: diltiazem hydrochloride was dissolved in sat. $NaHCO_3$ aq, extracted by chloroform, washed with sat NaCl aq, and dried before VCD measurement; similarly, penicillin G sodium salt was dissolved in 1 N HCl aq., extracted by chloroform, and dried before VCD measurement.

Computation:

MMFF MonteCarlo search was performed on SPARTAN'10 software[1], and density functional theory calculation was carried out on Gaussian 03 package[2]. All calculation was conducted without considering solvent effects.

Prior to the calculation of vibrational spectra of (*S*)-**1c**, a preliminary conformational search based on MMFF calculation using Spartan program was attempted. However, this method did not yield the expected *s*-trans conformation of the ester bond as a stable conformation; moreover, it failed to take into account the isomerism of the butyrolactone ring[3]. Therefore, several conformers of (*S*)-**1c** that differ in the conformation of the five-membered ring and the dihedral angles of the Hα-Cα-Oα-C_{Ac} (ϕ) and Cα-Oα-C_{Ac}=O_{Ac} (ψ) were manually generated, and submitted to DFT optimization at the level of B3LYP/6-311++G(d,p). Two conformers, (*S*)-**1c**-1 (ΔE = 0.000 kcal/mol) and (*S*)-**1c**-2 (ΔE = 0.886 kcal/mol) were obtained within 1.5 kcal/mol from the most stable conformer (Figure S2), and were taken into account for VCD and IR calculation. The ester conformations of the two stable conformers were close to *s*-trans, and their ester carbonyl groups are in a *syn* relationship with the methine hydrogen (Figure S2), which bolstered the stability of the assumed dominant conformation of esters. The third lowest-energy conformer exhibited an *s*-cis ester conformation, and was less stable than (*S*)-**1c**-1 by 1.771 kcal/mol, which also supported the preferred conformation of ester bond be *s*-trans. Boltzmann populations of (*S*)-**1c**-1 and (*S*)-**1c**-2 were calculated at 298 K. The VCD and IR spectra of each conformer were calculated at the DFT/B3LYP/6-311++G(d,p) level, and simulated with Lorentzian lineshapes of 8 cm^{-1} width. The calculated frequencies ν were scaled with the equation of $0.9894\nu - 0.0000104\nu^2$. Final spectra were obtained based on the Boltzmann population average of each spectrum (Figure S2). VCD and IR calculation of **2c** were carried out in a similar manner, and presented in Figure S2.

As expected from the results of the MMFF conformational search of (*S*)-**1c**, this method underestimated the stability of *s*-trans ester conformers in some cases. Therefore, the most stable conformers of **1**-**9** were calculated either by a MMFF MonteCarlo search with fixing ϕ and ψ at 0°, or by a MMFF conformational search without such fixation and the following DFT optimization using the B3LYP/6-31G(d) or B3LYP/6-311++G(d,p) level of all MMFF-generated conformers within 15 kJ/mol from the most stable. The ester conformations of the DFT-predicted most stable conformers of each molecule were close to that expected from the *s*-trans ester conformation and the *syn* relationship between the ester carbonyl and the methine hydrogen.

Supporting Figures

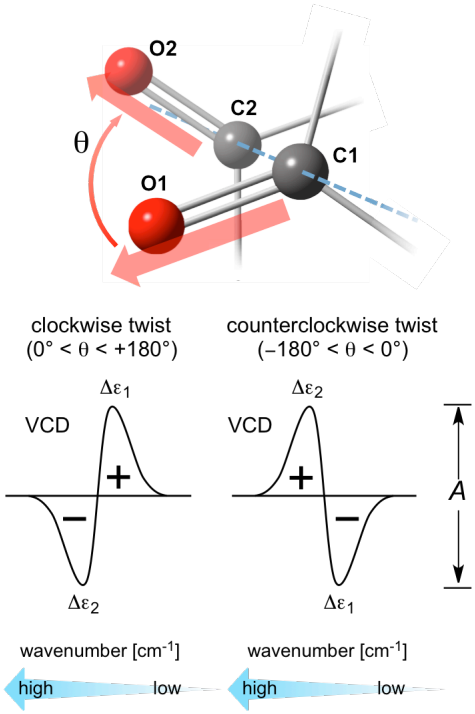


Figure S1. The illustrative representation of the definition of θ (the dihedral angle of two C=O groups, defined by O1-C1-C2-O2), $\Delta\varepsilon_1$ (the first Cotton effect), $\Delta\varepsilon_2$ (the second Cotton effect), and *A* (the amplitude of the couplet).

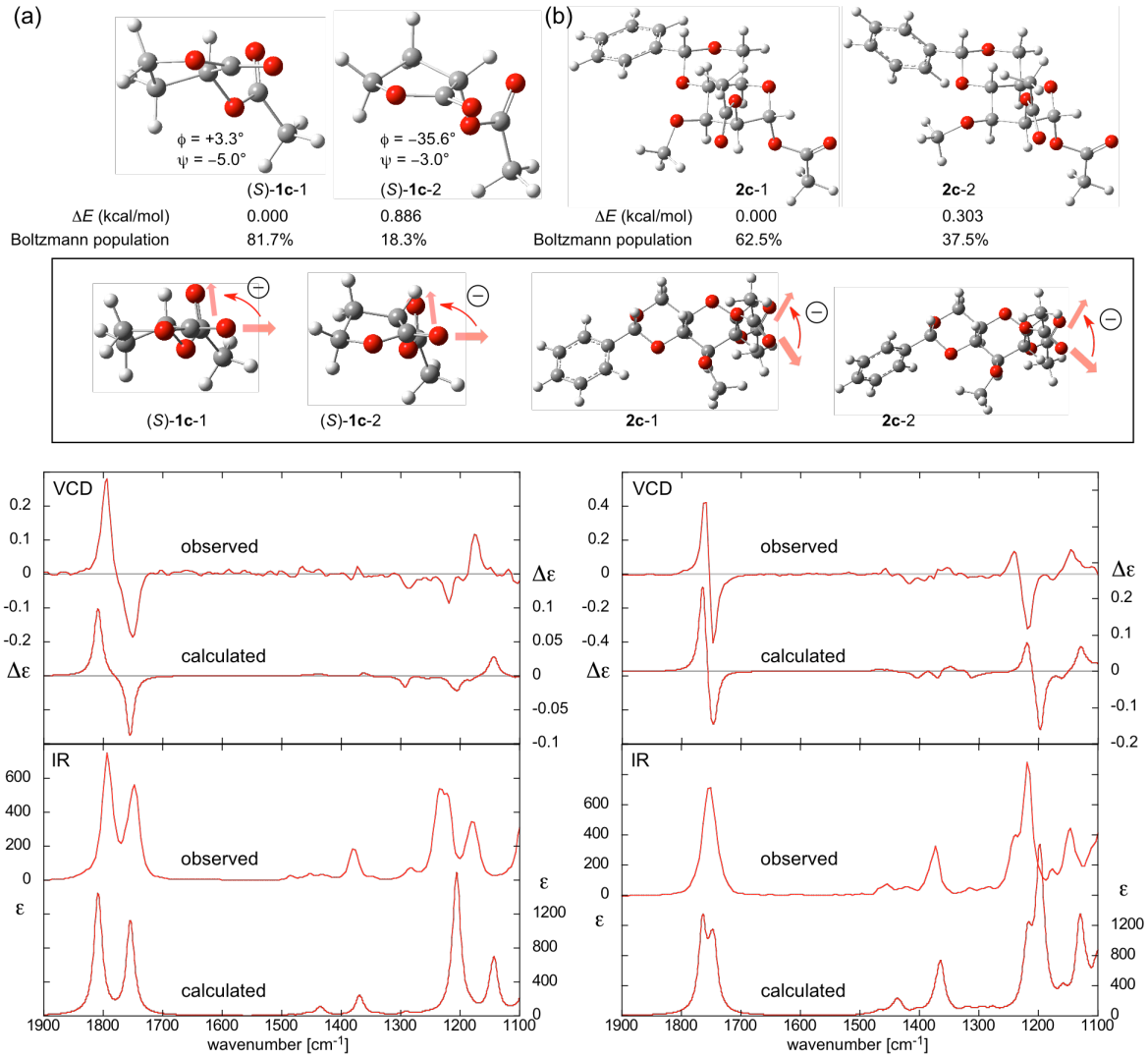


Figure S2. The accurate prediction of the VCD coupling by DFT calculation. The stable conformers of (a) (*S*)-**1c** and (b) **2c** calculated by DFT method confirmed the expected stability of the *s*-trans ester conformation with the carbonyl and the methine hydrogen in the *syn* relationship. The absolute twist of each compound, roughly seen from one carbonyl carbon to the other, was shown in the middle. The observed and calculated vibrational spectra are compared. The measurement condition is described in the caption of Figure 1, while the calculation condition is in "Computation" section. ϕ = dihedral angle of the Hα-Cα-Oα-C_{Ac}, ψ = dihedral angle of Cα-Oα-C_{Ac}=O_{Ac}.

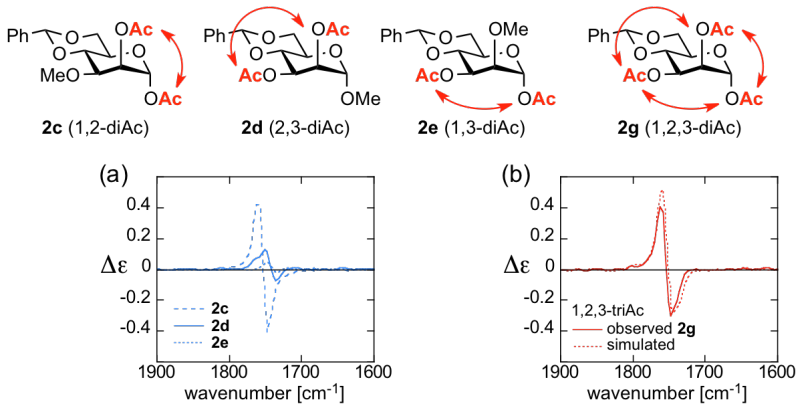


Figure S3. The validity of the addivity rule in VCD exciton chirality method. (a) The observed VCD spectra of **2c**, **2d** and **2e** and (b) the comparison of the observed VCD spectrum of **2g** and the simulated spectrum obtained by the sum of the observed VCD spectra of **2c**, **2d** and **2e**. The VCD couplet of a trischromophoric system (**2g**) was satisfactorily reproduced by the sum of the observed spectra of the component bischromophoric systems (**2c**, **2d** and **2e**). The VCD spectra were measured using $CDCl_3$ (c = 0.05 M, l = 100 μm) for 90 min, and corrected by a solvent spectrum obtained in the same measurement condition.

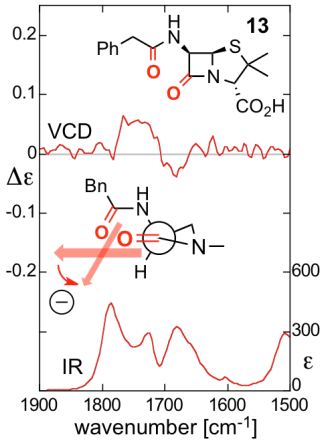


Figure S4. The VCD (top) and IR (bottom) spectra and the arrangement of two carbonyl chromophores of penicillin G **13**. The IR and VCD spectra were measured for 2 and 90 mins, respectively, in $CDCl_3$ (l = 100 μm) at a concentration of ca. 0.02 M. Each spectrum was corrected by a solvent spectrum obtained under the identical measurement condition.

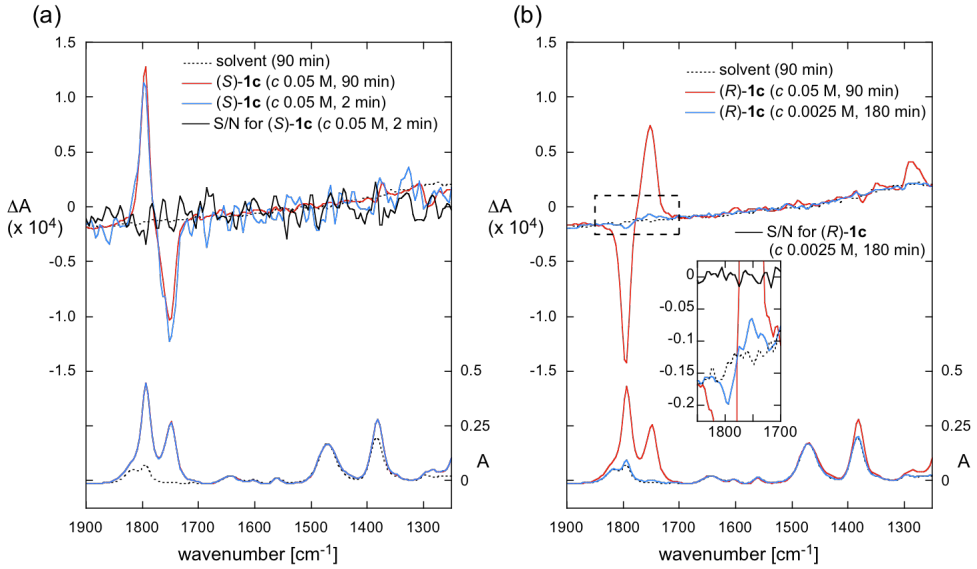
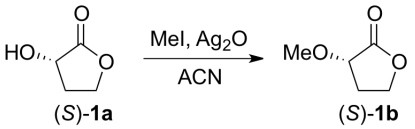


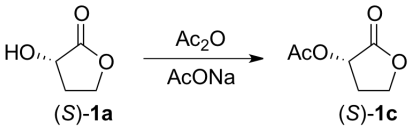
Figure S5. Application of VCD exciton chirality method to (a) the 2-min measurement and (b) the low-concentration measurement. The measurement was conducted using $CDCl_3$ in a CaF_2 cell with a 100-μm pathlength, and presented without solvent subtraction. The region in the dashed-line in (b) is magnified in the inset.

Preparation



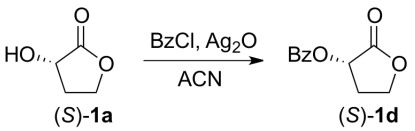
(*S*)-**1a** (*S*)-**1b**

(*S*)-α-methoxy-γ-butyrolactone ((*S*)-1b): The synthesis of (*S*)-**1b** was carried out using a similar procedure reported in ref 4. (*S*)-**1a** (102 mg, 1.00 mmol) in acetonitrile (2.5 mL) was added methyl iodide (249 μL, 4.0 mmol) and silver (I) oxide (288 mg, 1.24 mmol), and the resultant mixture was refluxed at 75 C° overnight in the absence of light (aluminum foil cover). The mixture was filtered through Celite, and the filter rinsed with diethyl ether. The filtrated was concentrated under reduced pressure. The residue was purified by column chromatography (hexane:EtOAc = 4:1- 3:1), affording (*S*)-**1b** (32 mg, 28%). The NMR spectrum was virtually identical with that reported in ref 4. $[\alpha]_D$ -75.3 (*c* 0.1, $CHCl_3$).



(*S*)-**1a** (*S*)-**1c**

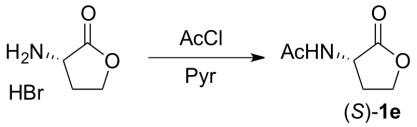
(*S*)-α-acetoxy-γ-butyrolactone ((*S*)-1c): (*S*)-**1a** (102 mg, 1.00 mmol) was added acetic anhydride (0.5 mL) and sodium acetate (7.8 mg, 0.095 mmol) and refluxed at 60 C°. After 1 h, the mixture was cooled to rt and then added to diethyl ether and half-saturated $NaHCO_3$ aq. The organic phase was separated, washed once with sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the crude mixture was purified by column chromatography (hexane:EtOAc = 3:2 - 0:1), affording (*S*)-**1c** (47 mg, 33%). The NMR spectrum was identical with that reported in ref 5. $[\alpha]_D$ -20.1 (*c* 0.3, $CHCl_3$); lit[5] $[\alpha]_D$ -20.7 (*c* 0.9, $CHCl_3$).



(*S*)-**1a** (*S*)-**1d**

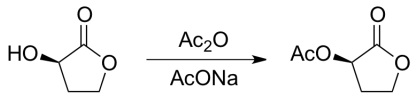
(*S*)-α-benzoyloxy-γ-butyrolactone ((*S*)-1d): (*S*)-**1a** (104 mg, 1.02 mmol) in dichloromethane 3 mL was added benzoyl chloride (173 μL, 1.5 mmol) and silver (I) oxide (288 mg, 1.24 mmol), and the resultant mixture was refluxed at 40 C° overnight in the absence of light (aluminum foil

cover). The mixture was filtered through Celite, and the filter rinsed with diethyl ether. The filtrated was concentrated under reduced pressure. The residue was purified by column chromatography (hexane:EtOAc = 4:1- 3:1), affording (*S*)-**1d** (145 mg, 69%). ^{1}H NMR ($CDCl_3$) δ 8.08 (m, ArH, 2H), 7.60 (m, ArH, 1H), 7.46 (m, ArH, 2H), 5.65 (dd, Hα, *J* = 8.5, 9.5 Hz, 1H), 4.55 (m, Hγ, 1H), 4.37 (m, H'γ, 1H), 2.84 (m, Hβ, 1H), 2.44 (m, H'β, 1H); ^{13}C NMR ($CDCl_3$) δ 172.6 (lactone CO), 165.4 (O<u>C</u>OPh), 133.8 (Ar), 130.0 (Ar), 128.7 (Ar), 128.5 (Ar), 68.1 (Cα), 65.1 (Cγ), 29.2 (Cβ); HRMS (EI) *m/z* calcd for $C_{11}H_{10}O_4$ $[M]^+$ 206.0579, found 206.0577; $[\alpha]_D$ +15.2 (*c* 0.1, $CHCl_3$).



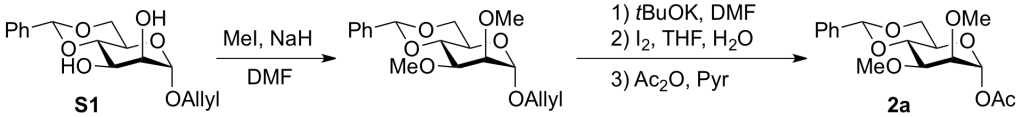
(*S*)-**1e**

(*S*)-α-acetamide-γ-butyrolactone ((*S*)-1e): (*S*)-(−)-2-amino-4-butyrolactone hydrobromide (46 mg, 0.25 mmol) in methanol (1 mL) was added pyridine (60.5 μL, 0.75 mmol) and acetyl chloride (35.5 μL, 0.50 mmol) and stirred at rt. After 2.5 h, most of the solvent was removed under reduced pressure and the residue was submitted to column chromatography (hexane:ethanol = 5:1-2:1), affording (*S*)-**1e** (13 mg, 36%). The NMR spectrum was similar to that reported in ref 6 with a slight difference in the chemical shift of the amide exchangeable proton. ^{1}H NMR ($CDCl_3$) δ 6.28 (br s, NH, 1H), 4.59 (ddd, Hα, *J* = 6.1, 8.6, 11.6 Hz, 1H), 4.47 (ddd, Hγ , *J* = 1.0, 9.1, 9.1 Hz, 1H), 4.29 (ddd, H'γ , *J* = 5.8, 9.3, 11.4 Hz, 1H), 2.83 (dddd, Hβ , *J* = 1.2, 5.9, 8.6, 12.5 Hz, 1H), 2.16 (dddd, H'β , *J* = 8.8, 11.5, 11.5, 12.5 Hz, 1H), 2.06 (s, Ac, 3H); $[\alpha]_D$ +26.3 (*c* 0.1, $CHCl_3$).



(*R*)-**1a** (*R*)-**1c**

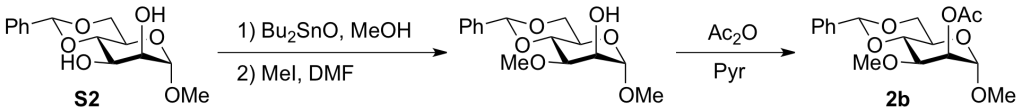
(*R*)-α-acetoxy-γ-butyrolactone ((*R*)-1c): Using a similar procedure for the synthesis of (*S*)-**1c**, (*R*)-**1c** was prepared from (*R*)-**1a** (102 mg, 1.00 mmol) in 35% yield (50 mg). The NMR spectrum was identical with that reported in ref 5. $[\alpha]_D$ +19.8 (*c* 0.1, $CHCl_3$); lit[5] $[\alpha]_D$ +20.2 (*c* 1.4, $CHCl_3$).



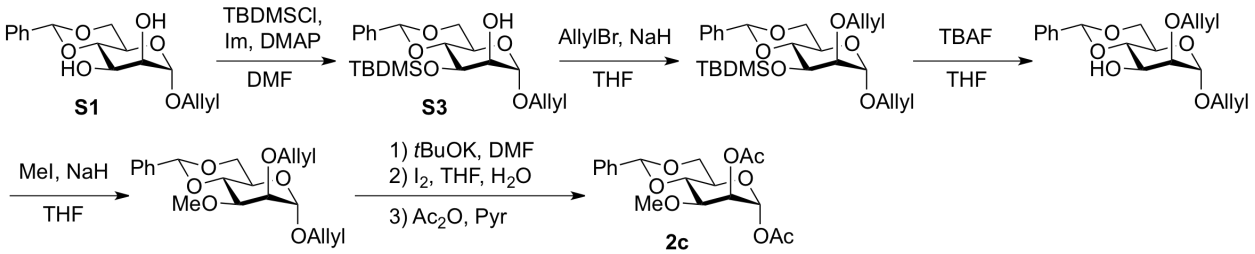
Acetyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-mannopyranoside (2a): **S1**[7] (136 mg, 0.441 mmol) in DMF (4 mL) was added sodium hydride (60% in oil, 44 mg) and stirred at 0 C°. After 10 min, the mixture was added methyl iodide 82.4 μL (1.32 mmol) and stirred at rt for 2 h. The mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq, and sat NaCl aq, and then dried over $MgSO_4$. After removal of the solvent, the crude mixture was purified by column chromatography (hexane:EtOAc = 6:1-3:1), affording the dimethyl mannoside (87 mg, 59%). Allyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-mannopyranoside: ^{1}H NMR ($CDCl_3$) δ 7.48 (m, ArH, 2H), 7.37-7.31 (m, ArH, 3H), 5.92 (m, CH_2=C<u>H</u>-, 1H), 5.59 (s, CHPh, 1H), 5.32 (m, C<u>H</u>H'=CH-, 1H), 5.24 (m, CH<u>H</u>'=CH-, 1H), 4.94 (d, H-1, *J* = 1.5 Hz, 1H), 4.24 (m, H-6a, 1H), 4.21 (m, -OC<u>H</u>H'-CH=, 1H), 4.07 (m, H-4, 1H), 4.00 (m, -OCH<u>H</u>'-CH=, 1H), 3.84-3.81 (m, H-5, H-6b, 2H), 3.76 (dd, H-3, *J* = 3.5, 9.9 Hz, 1H), 3.67 (dd, H-2, *J* = 1.8, 3.2 Hz, 1H), 3.56 (s, -OMe, 3H), 3.55 (s, OMe, 3H); ^{13}C NMR ($CDCl_3$) δ 137.5 (CH_2=<u>C</u>H-), 133.5 (Ar), 128.8 (Ar), 128.2 (Ar), 126.1 (Ar), 117.8 (<u>C</u>H_2=CH-), 101.6 (<u>C</u>HPh), 97.5 (C-1), 79.1 (C-4), 78.8 (C-2), 77.6 (C-3), 68.8 (C-6), 68.2 (-O<u>C</u>H_2-CH=), 64.0 (C-5), 59.7 (OMe), 59.1 (OMe); LRMS (ESI) *m/z* calcd for $C_{18}H_{24}NaO_6$ $[M+Na]^+$ 359.1, found 359.2; $[\alpha]_D$ +46.2 (*c* 0.1, $CHCl_3$).

The dimethyl mannoside (53 mg, 0.16 mmol) in DMF 1.5 mL was heated at 95 C° and added potassium *t*-butoxide (35 mg, 0.31 mmol). The mixture was stirred at the same temperature for 2.5 h. After cooling to rt, the mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, water, and sat NaCl aq, and then dried over $MgSO_4$. After removal of the solvent, the crude product was dissolved in THF (3.2 mL) and water (0.8 mL), added iodide (74 mg, 0.29 mmol), and stirred overnight at rt. The mixture was diluted with EtOAc, washed sequentially with 10% $Na_2S_2O_3$ aq and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture was briefly purified by column chromatography (hexane:EtOAc = 2:1-4:3), affording an anomeric mixture of deallyl mannoside (37 mg). Part of the product (27 mg) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight at rt. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 3.5:1), affording **2a** (22 mg, 49% in 3 steps) and a minor β-anomer component (1.2 mg). The anomeric

configuration of **2a** was determined by a NOE signal observed between H-1 and 2-OMe. This assignment was further corroborated by a NOE measurement of the β-anomer, which exhibited a NOE signal between H-1 and H-3. **2a**: ^{1}H NMR ($CDCl_3$) δ 7.48 (m, ArH, 2H), 7.38-7.32 (m, ArH, 3H), 6.19 (d, H-1, *J* = 1.7 Hz, 1H), 5.60 (s, CHPh, 1H), 4.27 (dd, H-6a, *J* = 4.2, 9.5 Hz, 1H), 4.14 (dd, H-4, *J* = 9.2, 9.8 Hz, 1H), 3.89-3.79 (m, H-5, H-6b, 2H), 3.74 (dd, H-3, *J* = 3.4, 10.0 Hz, 1H), 3.63 (dd, H-2, *J* = 1.9, 3.4 Hz, 1H), 3.59 (s, 3-OMe, 3H), 3.57 (s, 2-OMe, 3H), 2.15 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 169.0 (CO), 137.3 (Ar), 129.0 (Ar), 128.2 (Ar), 126.1 (Ar), 101.6 (CHPh), 91.5 (C-1), 78.6 (C-4), 77.8 (C-2), 77.2 (C-3), 68.5 (C-6), 66.1 (C-5), 59.7 (2-OMe), 59.1 (3-OMe), 21.1 ($COCH_3$); LRMS (ESI) *m/z* calcd for $C_{17}H_{22}NaO_7$ $[M+Na]^+$ 361.1, found 361.2; $[\alpha]_D$ +58.2 (*c* 0.1, $CHCl_3$).



Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (2b): A readily available **S2** was converted to the 3-OMe derivative using a similar procedure reported in ref 8. The 3-OMe mannoside (23 mg) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 3.5:1), affording pure fractions of **2b** (18 mg). ^{1}H NMR ($CDCl_3$) δ 7.50 (m, ArH, 2H), 7.38-7.32 (m, ArH, 3H), 5.61 (s, CHPh, 1H), 5.35 (dd, H-2, *J* = 1.7, 3.5 Hz, 1H), 4.69 (d, H-1, *J* = 1.7 Hz, 1H), 4.28 (m, H-6a, 1H), 3.99 (m, H-4, 1H), 3.88-3.82 (m, H-5, H-6b, 2H), 3.78 (dd, H-3, *J* = 3.8, 10.0 Hz, 1H), 3.45 (s, OMe, 3H), 3.40 (s, OMe, 3H), 2.16 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 170.2 (CO), 137.3 (Ar), 129.0 (Ar), 128.2 (Ar), 126.2 (Ar), 101.9 (CHPh), 99.7 (C-1), 78.5 (C-4), 75.8 (C-3), 69.1 (C-2), 68.8 (C-6), 63.7 (C-5), 58.4 (OMe), 55.1 (OMe), 21.0 ($COCH_3$); LRMS (ESI) *m/z* calcd for $C_{17}H_{22}NaO_7$ $[M+Na]^+$ 361.1, found 361.2; $[\alpha]_D$ +30.5 (*c* 0.1, $CHCl_3$).



Acetyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (2c): **S1** (375 mg, 1.22 mmol) in DMF (8 mL) was added imidazole (166 mg, 2.44 mmol), DMAP (15 mg, 0.12 mmol) and *t*-butyldimethylchlorosilane (212 mg, 1.41 mmol) and stirred at rt. After 4 h, the mixture diluted with EtOAc, washed sequentially with 5% citric acid aq, sat $NaHCO_3$ aq, and sat NaCl aq, and then dried over $MgSO_4$. After removal of the solvent, the crude mixture was purified by column chromatography (hexane:EtOAc = 12:1 - 8:1), affording **S3** (47 mg, 33%). **S3**: ^{1}H NMR ($CDCl_3$) δ 7.48 (m, ArH, 2H), 7.38-7.33 (m, ArH, 3H), 5.92 (m, CH_2=C<u>H</u>-, 1H), 5.55 (s, CHPh, 1H), 5.30 (m, C<u>H</u>H'=CH-, 1H), 5.23 (m, CH<u>H</u>'=CH-, 1H), 4.95 (d, H-1, *J* = 1.6 Hz, 1H), 4.26 (m, H-6a, 1H), 4.20 (m, -OC<u>H</u>H'-CH=, 1H), 4.09 (m, H-3, 1H), 4.02 (m, -OCH<u>H</u>'-CH=, 1H), 3.90 (dd, H-2, *J* = 0.8, 3.8 Hz, 1H), 3.88-3.78 (m, H-4, H-5, H-6b, 3H), 2.84 (s, 2-OH, 1H), 0.88 (s, *t*Bu, 9H), 0.11 (s, Me, 3H), 0.06 (s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 137.5 (Ar), 133.6 (CH_2=<u>C</u>H-), 128.8 (Ar), 128.1 (Ar), 126.1 (Ar), 117.8 (<u>C</u>H_2=CH-), 101.9 (<u>C</u>HPh), 98.9 (C-1), 79.1 (C-4), 72.0 (C-2), 69.8 (C-3), 68.8 (C-6), 68.2 (-O<u>C</u>H_2-CH=), 63.2 (C-5), 33.1 (<u>C</u>$(CH_3)_3$), 25.7 (C(<u>C</u>$H_3)_3$), 18.1 (Si<u>C</u>$(CH_3)_2$), -4.36 (Me), -5.03 (Me); LRMS (ESI) *m/z* calcd for $C_{22}H_{34}NaO_6Si$ $[M+Na]^+$ 445.2, found 445.4; $[\alpha]_D$ +20.5 (*c* 0.1, $CHCl_3$).

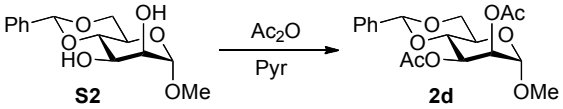
S3 (195 mg, 0.461 mmol) in THF (6 mL) was added sodium hydride (60% in oil, 37 mg) and stirred at 0 C°. After 20 min, the mixture was added allyl bromide 117 μL (1.38 mmol) and TBAI (17 mg) and stirred at rt for 4 h. The mixture was diluted with EtOAc, washed with water twice and with sat NaCl aq, and dried over $MgSO_4$. The crude product (224 mg) was used for the next reaction without further purification. Allyl 2-*O*-allyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside: ^{1}H NMR ($CDCl_3$) δ 7.48 (m, ArH, 2H), 7.37-7.32 (m, ArH, 3H), 5.98-5.87 (m, 2 x CH_2=C<u>H</u>-, 2H), 5.57 (s, CHPh, 1H), 5.29 (m, 2 x C<u>H</u>H'=CH-, 2H), 5.21 (m, 2 x CH<u>H</u>'=CH-, 2H), 4.84 (d, H-1, *J* = 1.8 Hz, 1H), 4.34 (m, -OC<u>H</u>H'-CH=, 1H), 4.23-4.15 (m, H-3, H-6a, -OC<u>H</u>H'-CH=, -OCH<u>H</u>'-CH=, 4H), 4.01-3.95 (m, H-4, -OCH<u>H</u>'-CH=, 2H), 3.85-3.75 (m, H-5, H-6b, 2H), 3.64 (dd, H-2, *J* = 1.6, 3.4 Hz, 1H), 0.88 (s, *t*Bu, 9H), 0.09 (s, Me, 3H), 0.04 (s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 137.7 (Ar), 135.0 (CH_2=<u>C</u>H-), 133.7 (CH_2=<u>C</u>H-), 128.8 (Ar),

128.0 (Ar), 126.2 (Ar), 117.5 ($\underline{C}H_2$=CH-), 117.5 ($\underline{C}H_2$=CH-), 101.9 ($\underline{C}$HPh), 99.0 (C-1), 79.2 (C-4), 79.0 (C-2), 73.6 (-O$\underline{C}H_2$-CH=), 70.4 (C-3), 68.8 (C-6), 68.0 (-O$\underline{C}H_2$-CH=), 64.3 (C-5), 33.1 ($\underline{C}(CH_3)_3$), 25.8 (C($\underline{C}H_3)_3$), 18.3 (Si$\underline{C}(CH_3)_2$), -4.4 (Me), -4.8 (Me); LRMS (ESI) *m/z* calcd for $C_{25}H_{39}O_6Si$ $[M+H]^+$ 463.3, found 463.4; $[\alpha]_D$ +66.0 (*c* 0.1, $CHCl_3$).

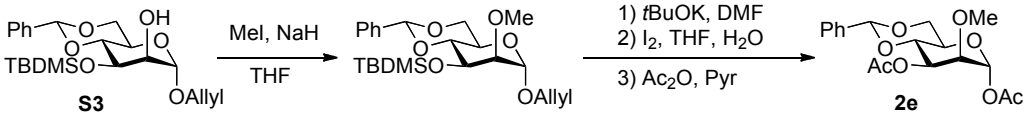
Part of the crude diallyl mannoside (204 mg) was dissolved in THF (6 mL) and added a THF solution of 1 M TBAF (1.13 mL) and stirred at rt. After 40 min, the mixture was diluted with EtOAc, washed sequentially with 5% citric acid aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. Removal of the solvent gave a crude product (159 mg) of the desilylated mannoside, whose spectroscopic data were identical with those reported in ref 9. Part of the crude desilylated mannoside (147 mg) was added THF (5 mL) and sodium hydride (60% in oil, 34 mg) and stirred at 0 C°. After 15 min, the mixture was added methyl iodide 66 μL and stirred at rt for 3.5 h. The mixture was diluted with EtOAc, washed sequentially with water and sat NaCl aq, and dried over $MgSO_4$. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 15:1 – 8:1), affording the 3-OMe mannoside (92 mg, 64% in 3 steps). Allyl 2-*O*-allyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside: ^{1}H NMR ($CDCl_3$) δ 7.49 (m, ArH, 2H), 7.38-7.31 (m, ArH, 3H), 5.98-5.87 (m, 2 x CH_2=C$\underline{H}$-, 2H), 5.60 (s, CHPh, 1H), 5.31 (m, 2 x C$\underline{H}$H'=CH-, 2H), 5.22 (m, 2 x CH$\underline{H}$'=CH-, 2H), 4.88 (d, H-1, *J* = 1.5 Hz, 1H), 4.28-4.14 (m, H-6a, 2 x -OC$\underline{H}$H'-CH=, -OCH$\underline{H}$'-CH=, 4H), 4.11 (m, H-4, 1H), 3.99 (m, -OCH$\underline{H}$'-CH=, 1H), 3.87-3.80 (m, H-2, H-5, H-6b, 3H), 3.75 (dd, H-3, *J* = 3.4, 10.0 Hz, 1H), 3.55 (s, OMe, 3H); ^{13}C NMR ($CDCl_3$) δ 137.6 (Ar), 134.7 (CH_2=$\underline{C}$H-), 133.5 (CH_2=$\underline{C}$H-), 128.8 (Ar), 128.2 (Ar), 126.1 (Ar), 117.7 ($\underline{C}H_2$=CH-), 117.7 ($\underline{C}H_2$=CH-), 101.6 ($\underline{C}$HPh), 98.4 (C-1), 79.1 (C-4), 77.8 (C-3), 75.8 (C-2), 72.9 (-O$\underline{C}H_2$-CH=), 68.8 (C-6), 68.1 (-O$\underline{C}H_2$-CH=), 64.1 (C-5), 59.0 (OMe); LRMS (ESI) *m/z* calcd for $C_{20}H_{26}NaO_6$ $[M+Na]^+$ 385.2, found 385.3; $[\alpha]_D$ +25.4 (*c* 0.1, $CHCl_3$).

The diallyl mannoside (64 mg, 0.18 mmol) in DMF 3 mL was heated at 95 C° and added potassium *t*-butoxide (79 mg, 0.70 mmol). The mixture was stirred at the same temperature for 2.5 h. After cooling to rt, the mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, water, and sat NaCl aq, and then dried over $MgSO_4$. After removal of the solvent, the crude product was dissolved in THF (4 mL) and water (1 mL), added iodide (135 mg, 0.53 mmol), and stirred overnight at rt. The mixture was diluted with EtOAc, washed sequentially with 10% $Na_2S_2O_3$ aq and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture

was briefly purified by column chromatography (hexane:EtOAc = 1:1 – 1:1.5), affording an anomeric mixture of deallyl mannoside (23 mg). Part of the product (21 mg) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight at rt. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 4.5:1), affording **2c** (12 mg, 20% in 3 steps). **2c**: ^{1}H NMR ($CDCl_3$) δ 7.50 (m, ArH, 2H), 7.39-7.35 (m, ArH, 3H), 6.05 (d, H-1, *J* = 1.8 Hz, 1H), 5.62 (s, CHPh, 1H), 5.36 (dd, H-2, *J* = 1.9, 3.4 Hz, 1H), 4.29 (dd, H-6a, *J* = 4.8, 10.1 Hz, 1H), 4.05 (dd, H-4, *J* = 9.5, 9.8 Hz, 1H), 3.90 (ddd, H-5, *J* = 4.5, 9.5, 10.0 Hz, 1H), 3.82 (dd, H-6b, *J* = 10.0, 10.5 Hz, 1H), 3.81 (dd, H-3, *J* = 3.5, 9.8 Hz, 1H), 3.49 (s, OMe, 3H), 2.18 (s, Ac, 3H), 2.17 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 169.8 (CO), 168.4 (CO), 137.1 (Ar), 129.1 (Ar), 128.3 (Ar), 126.1 (Ar), 101.9 (CHPh), 91.6 (C-1), 78.0 (C-4), 75.7 (C-3), 68.5 (C-6), 68.1 (C-2), 65.8 (C-5), 58.7 (OMe), 20.9 ($COCH_3$), 20.9 ($COCH_3$); LRMS (ESI) *m/z* calcd for $C_{18}H_{22}NaO_8$ $[M+Na]^+$ 389.1, found 389.3; $[\alpha]_D$ +43.1 (*c* 0.1, $CHCl_3$).



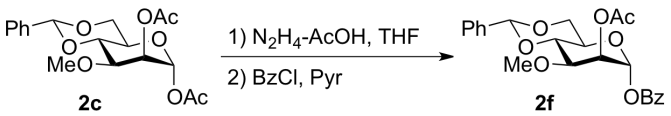
Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (2d): The synthesis of **2d** was carried out using pyridine and acetic anhydride as reported in ref 10.



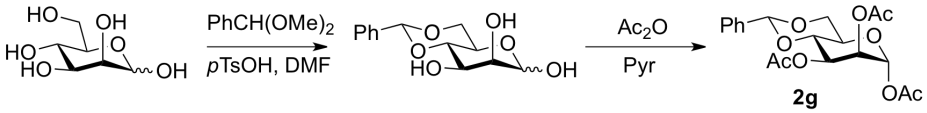
Acetyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranoside (2e): **S3** (100 mg, 0.237 mmol) in THF (3 mL) was added sodium hydride (60% in oil, 19 mg) and stirred at 0 C°. After 20 min, the mixture was added methyl iodide 37.4 μL and stirred at rt overnight. The mixture was diluted with EtOAc, washed with water twice and with sat NaCl aq, and dried over $MgSO_4$. The crude product (110 mg) was used for the next reaction without further purification. Allyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-*O*-methyl-α-D-mannopyranoside: ^{1}H NMR ($CDCl_3$) δ 7.48 (m, ArH, 2H), 7.37-7.32 (m, ArH, 3H), 5.96-5.88 (m, CH_2=C<u>H</u>-, 1H), 5.56 (s, CHPh, 1H), 5.30 (m, C<u>H</u>H'=CH-, 1H), 5.22 (m, CH<u>H</u>'=CH-, 1H), 4.88 (d, H-1, *J* = 1.8 Hz, 1H), 4.23-4.16 (m, H-3, H-6a, -OC<u>H</u>H'-CH=, 3H), 3.99 (m, -OCH<u>H</u>'-CH=, 1H), 3.93 (dd, H-4, *J*

= 9.2, 9.4 Hz, 1H), 3.85-3.75 (dd, H-6b, *J* = 9.4, 10.1 Hz, 1H), 3.78 (m, H-5, 1H), 3.59 (s, OMe, 3H), (dd, H-2, *J* = 1.6, 3.4 Hz, 1H), 0.89 (s, *t*Bu, 9H), 0.10 (s, Me, 3H), 0.05 (s, Me, 3H); ^{13}C NMR ($CDCl_3$) δ 137.6 (Ar), 133.6 (CH_2=$\underline{C}$H-), 128.7 (Ar), 128.0 (Ar), 126.1 (Ar), 117.5 ($\underline{C}H_2$=CH-), 101.8 ($\underline{C}$HPh), 98.5 (C-1), 81.9 (C-4), 79.2 (C-2), 70.5 (C-3), 68.8 (C-6), 68.0 (-O$\underline{C}H_2$-CH=), 64.3 (C-5), 60.8 (OMe), 33.1 ($\underline{C}(CH_3)_3$), 25.8 (C($\underline{C}H_3)_3$), 18.3 (Si$\underline{C}(CH_3)_2$), -4.4 (Me), -4.9 (Me); LRMS (ESI) *m/z* calcd for $C_{23}H_{36}NaO_6Si$ $[M+Na]^+$ 459.2, found 459.4; $[\alpha]_D$ +47.7 (*c* 0.1, $CHCl_3$).

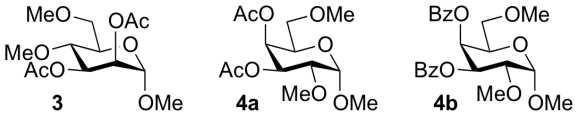
Part of the crude product (93 mg) was dissolved in DMF (3 mL) and heated at 95 C°. To this solution potassium *t*-butoxide (79 mg, 0.70 mmol) was added and stirred at the same temperature for 2.5 h. After cooling to rt, the mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, water, and sat NaCl aq, and then dried over $MgSO_4$, yielding a desilylated compound. After removal of the solvent, the crude product was dissolved in THF (4 mL) and water (1 mL), added iodide (101 mg), and stirred at rt for 3 h. The mixture was diluted with EtOAc, washed sequentially with 10% $Na_2S_2O_3$ aq and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture was briefly purified by column chromatography (hexane:EtOAc = 1:1 – 1:1.5), affording an anomeric mixture of di-1,3-OH mannoside (39 mg). Part of the product (20 mg) was added pyridine (1 mL) and acetic anhydride (0.5 mL) and stirred overnight at rt. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 4.5:1), affording **2e** (18 mg, 48% in 4 steps). **2e**: ^{1}H NMR ($CDCl_3$) δ 7.45 (m, ArH, 2H), 7.39-7.34 (m, ArH, 3H), 6.18 (d, H-1, *J* = 1.8 Hz, 1H), 5.57 (s, CHPh, 1H), 5.31 (dd, H-3, *J* = 3.4, 10.3 Hz, 1H), 4.28 (dd, H-6a, *J* = 4.9, 10.2 Hz, 1H), 4.15 (dd, H-4, *J* = 9.7, 10.3 Hz, 1H), 3.90 (ddd, H-5, *J* = 4.7, 9.6, 10.0 Hz, 1H), 3.82 (dd, H-6b, *J* = 10.3, 10.6 Hz, 1H), 3.73 (dd, H-2, *J* = 1.9, 3.6 Hz, 1H), 3.53 (s, OMe, 3H), 2.17 (s, Ac, 3H), 2.14 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4 (CO), 169.0 (CO), 137.1 (Ar), 129.1 (Ar), 128.3 (Ar), 126.1 (Ar), 101.9 ($\underline{C}$HPh), 91.2 (C-1), 77.6 (C-2), 75.6 (C-4), 70.1 (C-3), 68.5 (C-6), 66.2 (C-5), 59.8 (OMe), 21.1 ($\underline{C}OCH_3$), 21.0 ($\underline{C}OCH_3$); LRMS (ESI) *m/z* calcd for $C_{18}H_{22}NaO_8$ $[M+Na]^+$ 389.1, found 389.3; $[\alpha]_D$ +33.1 (*c* 0.1, $CHCl_3$).



Benzoyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-methyl-α-D-mannopyranoside (2f): **2c** (4.6 mg, 13 μmol) in THF (1 mL) was added hydrazine acetate (1.4 mg, 15 μmol) and stirred at 50 C°. After 3 h, the mixture was cooled to rt and then added to EtOAc and 5% citric acid aq. The organic phase was separated, washed with sat $NaHCO_3$ aq and then NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the crude product was added pyridine (0.25 mL) and benzoyl chloride (30 μL) and stirred at rt overnight. Most of the solvent was removed under reduced pressure, and the residue was submitted to column chromatography (hexane:EtOAc = 10:1 – 4:1), affording **2f** (1.4 mg, 25% in 2 steps). **2f**: ^{1}H NMR ($CDCl_3$) δ 8.07 (m, ArH, 2H), 7.64 (m, ArH, 1H), 7.50 (m, ArH, 4H), 7.39-7.35 (m, ArH, 3H), 6.32 (d, H-1, *J* = 2.0 Hz, 1H), 5.66 (s, CHPh, 1H), 5.22 (dd, H-2, *J* = 1.7, 3.5 Hz, 1H), 4.30 (dd, H-6a, *J* = 3.5, 10.6 Hz, 1H), 4.13 (dd, H-4, *J* = 9.7, 9.7 Hz, 1H), 4.03 (ddd, H-5, *J* = 4.9, 9.1, 9.7 Hz, 1H), 3.94 (dd, H-3, *J* = 3.6, 10.0 Hz, 1H), 3.86 (dd, H-6b, *J* = 10.3, 10.3 Hz, 1H), 3.54 (s, OMe, 3H), 2.22 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 169.8 (CO), 163.9 (CO), 137.1 (Ar), 134.0 (Ar), 130.0 (Ar), 129.1 (Ar), 128.8 (Ar), 128.7 (Ar), 128.3 (Ar), 126.1 (Ar), 101.8 (CHPh), 92.2 (C-1), 78.1 (C-4), 76.0 (C-3), 68.5 (C-6), 68.2 (C-2), 66.1 (C-5), 58.7 (OMe), 20.9 ($COCH_3$); LRMS (ESI) *m/z* calcd for $C_{23}H_{24}NaO_8$ $[M+Na]^+$ 451.1, found 451.3; $[\alpha]_D$ +59.2 (*c* 0.1, $CHCl_3$).



Acetyl di-2,3-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (2g): The synthesis of **2d** was carried out starting from D-mannose by using a similar procedure reported in ref 11.



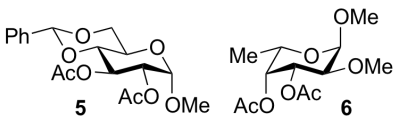
Methyl 2,3-di-*O*-acetyl-di-4,6-*O*-methyl-α-D-mannopyranoside (3), **Methyl 3,4-di-*O*-acetyl-di-2,6-*O*-methyl-α-D-mannopyranoside (4a)** , **Methyl 3,4-di-*O*-benzoyl-di-2,6-*O*-methyl-α-D-mannopyranoside (4b)**: Bisacyl compounds **3** and **4** were prepared by acylation of Methyl di-4,6-*O*-methyl-α-D-mannopyranoside[12] and Methyl di-2,6-*O*-methyl-α-D-mannopyranoside[13], respectively. Acetylation was conducted by pyridine and acetic anhydride, while benzoylation

was performed with benzoyl chloride and pyridine. After work-up, each bisacyl compound was obtained in 66-98% yield.

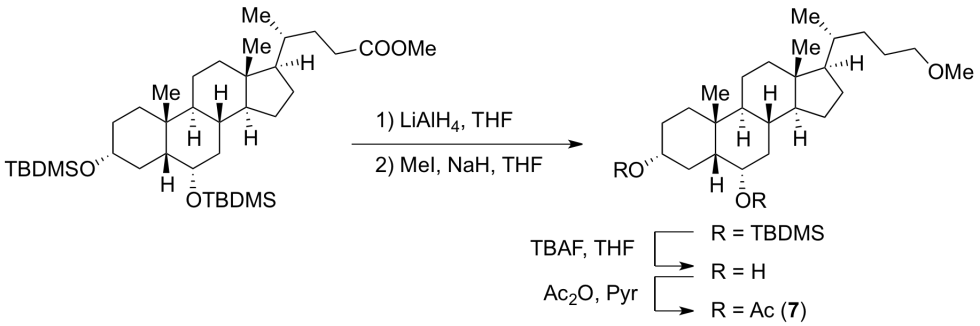
3: ^{1}H NMR ($CDCl_3$) δ 5.24-5.21 (m, H-2, H-3, 2H), 4.67 (d, H-1, *J* = 1.8 Hz, 1H), 3.71 (m, H-5, 1H), 3.67 (m, H-6a, 1H), 3.64 (m, H-4, 1H), 3.61 (dd, H-6b, *J* = 1.9, 10.5 Hz, 1H), 3.46 (s, OMe, 3H), 3.44 (s, OMe, 3H), 3.37 (s, OMe, 3H), 2.13 (s, Ac, 3H), 2.05 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 170.2 (CO), 169.9 (CO), 98.7 (C-1), 74.2 (C-4), 71.7 (C-3), 71.0 (C-6), 70.9 (C-5), 70.1 (C-2), 60.4 (OMe), 59.3 (OMe), 55.1 (OMe), 21.0 ($\underline{C}OCH_3$), 21.0 ($\underline{C}OCH_3$); LRMS (ESI) *m/z* calcd for $C_{13}H_{22}NaO_8$ $[M+Na]^+$ 329.1, found 329.2; $[\alpha]_D$ +52.3 (*c* 0.1, $CHCl_3$).

4a: ^{1}H NMR ($CDCl_3$) δ 5.43 (dd, H-4, *J* = 1.3, 3.3 Hz, 1H), 5.26 (dd, H-3, *J* = 3.4, 10.7 Hz, 1H), 4.98 (d, H-1, *J* = 3.4 Hz, 1H), 4.10 (m, H-5, 1H), 3.67 (dd, H-2, *J* = 3.6, 10.5 Hz, 1H), 3.55 (s, OMe, 1H), 3.46 (s, OMe, 1H), 3.46 (m, H-6a, 1H), 3.37 (dd, H-6b, *J* = 4.9, 10.1 Hz, 1H), 3.34 (s, OMe, 3H), 2.15 (s, Ac, 3H), 2.02 (s, Ac, 3H); ^{13}C NMR ($CDCl_3$) δ 170.2 (CO), 170.0 (CO), 97.9 (C-1), 75.7 (C-2), 71.3 (C-6), 70.0 (C-3), 69.3 (C-4), 67.3 (C-5), 59.3 (OMe), 59.0 (OMe), 55.5 (OMe), 20.9 ($\underline{C}OCH_3$), 20.7 ($\underline{C}OCH_3$); LRMS (ESI) *m/z* calcd for $C_{13}H_{22}NaO_8$ $[M+Na]^+$ 329.1, found 329.2; $[\alpha]_D$ +132 (*c* 0.1, $CHCl_3$).

4b: ^{1}H NMR ($CDCl_3$) δ 8.03 (m, ArH, 2H), 7.86 (m, ArH, 2H), 7.62 (m, ArH, 1H), 7.51-7.46 (m, ArH, 3H), 7.31 (m, ArH, 2H), 5.82 (dd, H-4, *J* = 1.3, 3.4 Hz, 1H), 5.65 (dd, H-3, *J* = 3.4, 10.3 Hz, 1H), 5.10 (d, H-1, *J* = 3.8 Hz, 1H), 4.31 (ddd, H-5, *J* = 1.0, 4.8, 6.8 Hz, 1H), 3.93 (dd, H-2, *J* = 3.5, 10.4 Hz, 1H), 3.56 (dd, H-6a, *J* = 7.0, 10.2 Hz, 1H), 3.53 (s, OMe, 1H), 3.49 (s, OMe, 1H), 3.48 (dd, H-6a, *J* = 4.8, 10.5 Hz, 1H), 3.32 (s, OMe, 3H); ^{13}C NMR ($CDCl_3$) δ 165.5 (CO), 165.4 (CO), 133.3 (Ar), 132.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 128.5 (Ar), 128.2 (Ar), 98.2 (C-1), 76.4 (C-2), 71.5 (C-6), 70.8 (C-3), 70.0 (C-4), 67.8 (C-5), 59.6 (OMe), 59.3 (OMe), 55.6 (OMe); LRMS (ESI) *m/z* calcd for $C_{23}H_{26}NaO_8$ $[M+Na]^+$ 453.2, found 453.3; $[\alpha]_D$ +202 (*c* 0.2, $CHCl_3$).



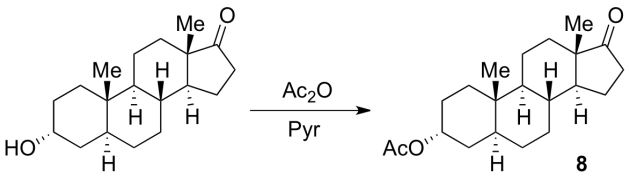
Methyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucopyranoside (5) , **Methyl 3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranoside (6)**: Compounds **5** and **6** were prepared by using a similar procedure reported in ref 14 and 15, respectively.



Di-3,6-acetyl steroid (7): Bis(*tert*-butyldimethylsilyl) hyodeoxycholic acid methyl ester[16] (234 mg, 0.368 mmol) in THF (3 mL) was added lithium aluminum hydride (31 mg, 0.82 mmol) at 0 C° and stirred at the same temperature for 30 min and then at rt for 5 h. The reaction mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq, sat potassium sodium tartrate aq and sat NaCl aq, and dried over $MgSO_4$, affording the crude corresponding alcohol (223 mg). Part of the crude product (191 mg) was added THF (5 mL) and sodium hydride (60% in oil, 24 mg) and stirred at 0 C°. After 10 min, the mixture was added methyl iodide (48 μL) and stirred at rt for 3 h. Methyl iodide (78 μL) was further added to the reaction mixture and stirred at rt overnight. Since unreacted starting material was still observed, the mixture was again added methyl iodide (48 μL) and heated to 90 C° for 5 h. The mixture was then diluted with EtOAc, washed sequentially with water and sat NaCl aq, and dried over $MgSO_4$. After dryness under reduced pressure, the crude product was submitted to column chromatography (hexane:EtOAc = 99:1 – 98:2), affording the methyl ether (129 mg, 66% in 2 steps). Di-3,6-*tert*-butyldimethylsilyl steroid methyl ether: ^{1}H NMR ($CDCl_3$) δ 3.97 (ddd, H-6, *J* = 4.2, 4.5, 11.7 Hz, 1H), 3.52 (m, H-3, 1H), 3.36-3.31 (m, 24-CH_2O, 2H), 3.33 (s, OMe, 3H), 1.94 (m, 1H), 1.90-1.79 (m, 2H), 1.73 (ddd, *J* = 3.0, 3.3, 14.3 Hz, 1H), 1.64 (m, 1H), 1.56-1.52 (m, 2H), 1.49-1.33 (m, 10H), 1.27-0.94 (m, 9H), 0.91 (d, 21-CH_3, J = 6.6 Hz, 3H), 0.89 (s, *t*Bu, 9H), 0.87 (s, *t*Bu, 9H), 0.87 (s, 19-CH_3, 3H), 0.63 (s, 18-CH_3, 3H), 0.05 (s, SiCMe, 3H), 0.05 (s, SiCMe, 3H), 0.03 (s, 2 x SiCMe, 6H); ^{13}C NMR ($CDCl_3$) δ 73.4 (24-CH_2O), 73.0 (3-CH), 68.7 (6-CH), 58.5 (OMe), 56.2, 56.1, 49.6, 42.8, 40.0, 39.6, 36.0, 35.9, 35.7, 35.4, 34.9, 32.2, 31.0, 29.8, 28.2, 26.3 (23-CH_2), 26.0 (C($\underline{C}H_3)_3$), 25.9 (C($\underline{C}H_3)_3$), 24.2, 23.5 (19-CH_3), 20.8, 18.6, 18.4, 18.1, 12.0 (18-CH_3), -4.5 (SiC$\underline{C}H_3$), -4.6 (SiC$\underline{C}H_3$), -4.8 (SiC$\underline{C}H_3$), -4.8 (SiC$\underline{C}H_3$); LRMS (ESI) *m/z* calcd for $C_{37}H_{72}NaO_3Si_2$ $[M+Na]^+$ 643.5, found 643.5; $[\alpha]_D$ +6.4 (*c* 0.1, $CHCl_3$).

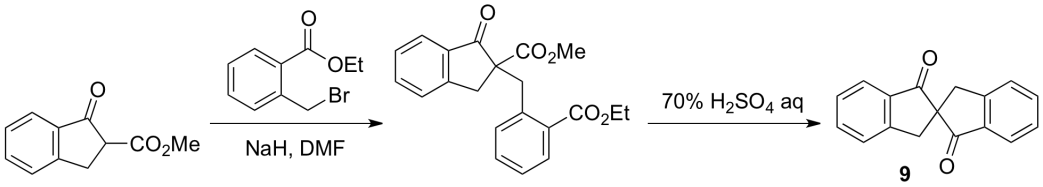
The methyl ether (98 mg, 0.16 mmol) in THF (3 mL) was added a THF solution of 1 M TBAF (0.4 mL) and stirred at rt overnight. The mixture was diluted with EtOAc, washed sequentially with 5% citric acid aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture was submitted to column chromatography (hexane:EtOAc = 1:3 – 1:5), affording the desilylated steroid (45 mg, 73%). Di-3,6-ol steroid methyl ether: ^{1}H NMR ($CDCl_3$) δ 4.05 (ddd, 6-H, *J* = 4.8, 4.8, 11.9 Hz, 1H), 3.61 (m, 3-H, 1H), 3.36-3.31 (m, 24-CH_2O, 2H), 3.33 (s, OMe, 3H), 1.98 (m, 1H), 1.93-1.80 (m, 2H), 1.79 (ddd, *J* = 3.0, 3.3, 14.2 Hz, 1H), 1.72-1.55 (m, 5H), 1.48-1.28 (m, 8H), 1.26 (m, 1H), 1.24-1.01 (m, 8H), 0.92 (d, 21-CH_3, *J* = 6.4 Hz, 3H), 0.91 (s, 19-CH_3, 3H), 0.64 (s, 18-CH_3, 3H); ^{13}C NMR ($CDCl_3$) δ 73.5 (24-CH_2O), 71.6 (3-CH), 68.1 (6-CH), 58.5 (OMe), 56.2, 56.2, 48.5, 42.8, 40.0, 39.9, 36.0, 35.6, 35.6, 35.0, 34.9, 32.2, 30.2, 29.2, 28.2, 26.2, 24.2, 23.5 (19-CH_3), 20.8, 18.6 (21-CH_3), 12.0 (18-CH_3); LRMS (ESI) *m/z* calcd for $C_{25}H_{44}NaO_3$ $[M+Na]^+$ 415.3, found 415.3; $[\alpha]_D$ +12.1 (*c* 0.1, $CHCl_3$).

The diol (17 mg, 43 μmol) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight at rt. After dryness under reduced pressure, the crude product was dissolved in EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. The removal of the solvent afforded **7** (18 mg, 88%). **7**: ^{1}H NMR ($CDCl_3$) δ 5.15 (ddd, 6-H, *J* = 4.6, 4.9, 12.5 Hz, 1H), 4.70 (m, 3-H, 1H), 3.37-3.30 (m, 24-CH_2O, 2H), 3.33 (s, OMe, 3H), 2.04 (s, Ac, 3H), 2.01 (s, Ac, 3H), 1.99 (m, 1H), 1.89-1.78 (m, 3H), 1.78-1.58 (m, 5H), 1.57-1.50 (m, 6H), 1.50-1.36 (m, 6H), 1.32-1.02 (m, 9H), 0.97 (s, 19-CH_3, 3H), 0.92 (d, 21-CH_3, *J* = 6.4 Hz, 3H), 0.64 (s, 18-CH_3, 3H); ^{13}C NMR ($CDCl_3$) δ 170.5 (CO), 170.5 (CO), 73.7 (3-CH), 73.5 (24-CH_2O), 71.0 (6-CH), 58.5 (OMe), 56.2, 56.2, 45.4, 42.9, 39.9, 39.9, 36.1, 35.6, 35.1, 34.6, 32.2, 31.3, 28.2, 26.5, 26.3, 26.2, 24.1, 23.3 (19-CH_3), 21.4 ($\underline{C}OCH_3$), 21.4 ($\underline{C}OCH_3$), 20.7, 18.6 (21-CH_3), 12.0 (18-CH_3); LRMS (ESI) *m/z* calcd for $C_{29}H_{48}NaO_5$ $[M+Na]^+$ 499.3, found 499.3; $[\alpha]_D$ +18.8 (*c* 0.1, $CHCl_3$).



3-*O*-Acetyl-*cis*-androsterone (8): *cis*-Androsterone (21.2 mg, 73 μmol) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight at rt. After dryness under reduced

pressure, the crude product was dissolved in EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. The removal of the solvent afforded **8** (24.3 mg, quant.), whose spectroscopic data were reported in ref 17.

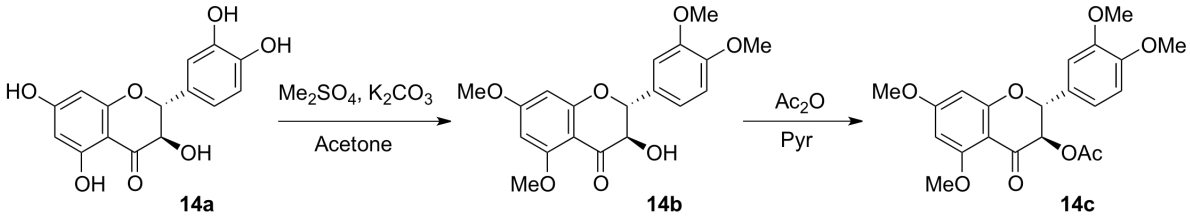


2,2′-spirobiindane-1,1′-dione (9): The synthesis of methyl 1-indanone-2-carboxylate and ethyl 2-(bromomethyl)benzoate was carried out by using similar procedures reported in refs 18 and 19, respectively. Methyl 1-indanone-2-carboxylate (60 mg, 0.32 mmol) in DMF (1 mL) was added dropwisely to a stirred solution of sodium hydride (60% in oil, 17 mg) in DMF (1 mL) at rt, then the reaction temperature was raised to 60 C°. After 30 min, ethyl 2-(bromomethyl)benzoate (76 mg, 0.31 mmol) in DMF (1 mL) was added dropwisely, and the mixture was stirred at 60 C° overnight. The mixture was diluted with Et_2O, washed with water and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture was submitted to column chromatography (hexane:EtOAc = 4:1), affording the diester (88 mg, 80%). 2-Methoxycarbonyl-2-(2-(ethoxycarbonyl)phenylmethyl)-1-indanone: ^{1}H NMR ($CDCl_3$) δ 7.81 (m, ArH, 1H), 7.74 (m, ArH, 1H), 7.52 (ddd, ArH, *J* = 1.1, 7.5, 7.5 Hz, 1H), 7.32 (m, ArH, 2H), 7.26 (m, ArH, 1H), 7.20 (m, ArH, 2H), 4.31 (m, -C<u>H$_2$</u>CH$_3$, 2H), 4.09 (d, -C<u>H</u>H'-, *J* = 14.4 Hz, 1H), 3.70 (s, $COOCH_3$, 3H), 3.69 (d, -CH<u>H</u>'-, *J* = 14.4 Hz, 1H), 3.61 (d, -C<u>H</u>H'-, *J* = 17.5 Hz, 1H), 3.09 (d, -CH<u>H</u>'-, *J* = 17.3 Hz, 1H), 1.34 -CH$_2$C<u>H$_3$</u>, 3H); ^{13}C NMR ($CDCl_3$) δ 202.6 (ketone CO), 171.4 (<u>C</u>OOMe), 167.8 (<u>C</u>OOEt), 153.7 (Ar), 137.8 (Ar), 135.2 (Ar), 135.1 (Ar), 131.7 (Ar), 131.5 (Ar), 131.3 (Ar), 130.5 (Ar), 127.5 (Ar), 126.7 (Ar), 126.2 (Ar), 124.6 (Ar), 61.8 (C-2), 61.1 (-<u>C</u>H$_2$CH$_3$), 52.9 (Me), 35.8 (-CH$_2$-), 35.6 (-CH$_2$-), 14.2 (-CH$_2$<u>C</u>H$_3$); LRMS (ESI) *m/z* calcd for $C_{21}H_{20}NaO_5$ $[M+Na]^+$ 375.1, found 375.2.

The diester (50 mg, 0.14 mmol) was added 70% H_2SO_4 aq and stirred at 90 C°. After 2 h, the mixture was cooled to rt, added ice-cold half-saturated $NaHCO_3$ aq dropwisely, and extracted with chloroform. The organic phase was separated, washed once with sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the crude mixture was purified by column chromatography (hexane:EtOAc = 5:1), affording (±)-**9** (35 mg, 60%). The NMR spectrum was identical with that

reported in ref 20.

The enantiomers of **9** were separated by chiral HPLC on a CHIRALPAK IA column (1 cm ϕ x 2 cm, and 1 cm ϕ x 25 cm) at hexane:ethanol = 85:15. The levorotatory enantiomer was eluted at t_1 = 7.2 min, while the dextrorotatory one appeared at t_2 = 9.3 min. The separation factor α was calculated as 1.4, where t_0 = 1.8 min was used. Baseline separation was observed for an injection of 4 mg of **9**. **(*aR*)-(–)-9**: $[\alpha]_D$ -151.5 (*c* 0.1, $CHCl_3$); lit[21] $[\alpha]_D$ -162 (*c* not described, $CHCl_3$). **(*aS*)-(+)-9**: $[\alpha]_D$ +150.7 (*c* 0.1, $CHCl_3$); lit[20] $[\alpha]_D$ +147.4 (*c* 1.25, $CHCl_3$).



(2*R*,3*R*)-3-*O*-acetyl-5,7,3′,4′-tetra-*O*-methyltaxifolin (14c): (+)-Taxifolin (23.5 mg, 77 μmol) in acetone (5 mL) was added K_2CO_3 (85 mg) and dimethyl sulfate (73 μL) and stirred at 70 C°. After 5 acetic anhydride (0.25 mL) and stirred at rt for 5 h. The reaction mixture was diluted with EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. After removal of the solvent, the mixture was submitted to column chromatography (hexane:EtOAc = 2:1 – 2:3), affording **14b** (8.2 mg, 29%). A more efficient tetramethylation of taxifolin using diazomethane was reported in ref 22. **14b**: ^{1}H NMR ($CDCl_3$) δ 7.12 (dd, H-6′, *J* = 2.0, 8.0 Hz, 1H), 7.08 (d, H-2′, *J* = 1.7 Hz, 1H), 7.08 (d, H-5′, *J* = 8.4 Hz, 1H), 6.15 (d, H-6, *J* = 2.3 Hz, 1H), 6.12 (d, H-8, *J* = 2.3 Hz, 1H), 4.98 (d, H-2, *J* = 12.1 Hz, 1H), 4.46 (dd, H-3, *J* = 2.0, 12.2 Hz, 1H), 4.07 (d, OH-3, *J* = 1.7 Hz, 1H), 3.94, 3.93, 3.91, 3.83 (s x 4, OMe x 4, 3H x 4); ^{13}C NMR ($CDCl_3$) δ 190.8 (C-4), 167.0 (C-5), 165.0 (C-8a), 162.2 (C-7), 149.8 (C-4′), 149.2 (C-3′), 128.9 (C-1′), 120.4 (C-6′), 111.1 (C-5′), 110.3 (C-2′), 102.9 (C-4a), 93.7 (C-6), 93.3 (C-8), 83.3 (C-2), 72.6 (C-3), 56.5, 56.0, 55.9, 55.8 (OMe x 4); $[\alpha]_D$ –24.7 (*c* 0.1, $CHCl_3$); lit[22] $[\alpha]_D$ –25.5 (*c* 1.13, $CHCl_3$).

The tetramethyl ether **14b** (5.2 mg, 14 μmol) was added pyridine (0.5 mL) and acetic anhydride (0.25 mL) and stirred overnight at rt. After dryness under reduced pressure, the crude product was dissolved in EtOAc, washed sequentially with 1 M HCl aq, sat $NaHCO_3$ aq and sat NaCl aq, and dried over $MgSO_4$. The removal of the solvent afforded **14c** (5.7 mg, quant). **14c**:

^{1}H NMR ($CDCl_3$) δ 7.03 (dd, H-6′, *J* = 2.1, 8.5 Hz, 1H), 7.00 (d, H-2′, *J* = 2.0 Hz, 1H), 6.89 (d, H-5′, *J* = 8.4 Hz, 1H), 6.14 (d, H-6, *J* = 2.3 Hz, 1H), 6.12 (d, H-8, *J* = 2.3 Hz, 1H), 5.71 (d, H-3, *J* = 12.1 Hz, 1H), 5.30 (d, H-2, *J* = 12.1 Hz, 1H), 3.91, 3.91, 3.89, 3.82 (s x 4, OMe x 4, 3H x 4); ^{13}C NMR ($CDCl_3$) δ 185.0 (C-4), 169.5 (CO) 166.5 (C-5), 164.2 (C-8a), 162.5 (C-7), 149.8 (C-4′), 149.1 (C-3′), 128.1 (C-1′), 120.5 (C-6′), 111.0 (C-5′), 110.2 (C-2′), 104.3 (C-4a), 93.6 (C-8), 93.5 (C-6), 81.1 (C-2), 73.5 (C-3), 56.2, 56.0, 55.9, 55.7 (OMe x 4); LRMS (ESI) *m*/*z* calcd for $C_{21}H_{22}O_8$ $[M+Na]^+$ 403.1, found 403.3; $[\alpha]_D$ +11.0 (*c* 0.1, $CHCl_3$).

References

1. SPARTAN'10, Wavefunction, Inc., Irvine, CA; www.wavefun.com/products/spartan.html
2. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision C.02, Gaussian, Inc., Wallingford CT, 2004.
3. Polavarapu, P. L.; Donahue, E. A.; Shanmugam, G.; Scalmani, G.; Hawkins, E. K.; Rizzo, C.; Ibnusaud, I.; Thomas, G.; Habel, D.; Sebastian, D. *J. Phys. Chem. A* **2011**, *115*, 5665-5673.
4. (a) Tanasova, M.; Yang, Q.; Olmsted, C. C.; Vasileiou, C.; Li, X.; Anyika, M.; Borhan, B. *Eur. J. Org. Chem.* **2009**, *2009*, 4242-4253. (b) Chen, Q.; Schweitzer, D.; Kane, J.; Davisson, V. J.; Helquist, P. *J. Org. Chem.* **2011**, *76*, 5157-5169.
5. Gotoh, M.; Barnes, C. N.; Kovác, P. *Carbohydr. Res.* **1994**, *260*, 203-218.
6. (a) Chhabra, S. R.; Stead, P.; Bainton, N. J.; Salmond, G. P. C.; Stewart, G. S. A. B. Williams,

P.; Bycroft, B. W. *J. Antibiotics* **1993**, *46*, 441-454. (b) Alferiev, I. S.; Hinson, J. T.; Ogle, M.; Breuer, E.; Levy, R. J. *Biomaterials* **2001**, *22*, 2501-2506.

7. Ogawa, T.; Kaburagi, T. *Carbohydr. Res*. **1982**, *110*, C12-C15.
8. (a) Nashed, M. A.; *Carbohydr. Res*. **1978**, *60*, 200-205. (b) Hirooka, M.; Terayama, M.; Mitani, E.; Koto, S.; Miura, A.; Chiba, K.; Takabatake, A.; Tashiro, T. Bull. Chem. Soc. Jpn. 2002, 75, 1301-1309.
9. Winnik, F. M.; Carver, J. P.; Krepinsky, J. J. *J. Org. Chem*. **1982**, *47*, 2701-2707.
10. Horton, D.; Luetzow, A. E. *Carbohydr. Res*. **1968**, *7*, 101-105.
11. Nóbrega, C.; Vázquez, J. T. *Tetrahedron Asymm*. **2003**, *14*, 2793-2801.
12. Khan, S. H.; Matta, K. L. *Carbohydr. Res*. **1993**, *243*, 29-42.
13. Rao, A. S; Roy, N. *Carbohydr. Res*. **1977**, *59*, 393-401.
14. Jeanloz, R. W.; Jeanloz, D. A. *J. Am. Chem. Soc*. **1957**, *79*, 2579-2583.
15. Dejter-Juszynski, M.; Flowers, H. M. *Carbohydr. Res*. **1973**, *79*, 2579-2583.
16. Ikegawa, S.; Onodera, H.; Kinoshita, J.; Tohma, M. *Chem. Pharm. Bull*. **1988**, *36*, 994-999.
17. Marwah, P.; Marwah, A.; Lardy, H. A. *Tetrahedron* **2003**, *59*, 2273-2287.
18. Van Emelen, K.; De Wit, T.; Hoornaert, G. J.; Compernolle, F. *Org. Lett*. **2000**, *2*, 3083-3086.
19. Anzalone, L.; Hirsch, J. A. *J. Org. Chem*. **1985**, *50*, 2128-2133.
20. Nieman, J.; Keay, B. A. *Tetrahedron Asymm*. **1995**, *6*, 1575-1583.
21. Meyer, A.; Neudeck, H.; Schlögl, K. *Tetrahedron Lett*. **1976**, *26*, 2233-2236.
22. Takahashi, H.; Li, S.; Harigaya, Y.; Onda, M. *J. Nat. Prod*. **1988**, *51*, 730-735.

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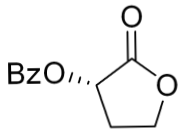

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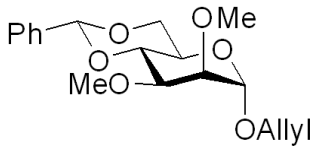

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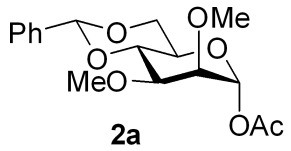

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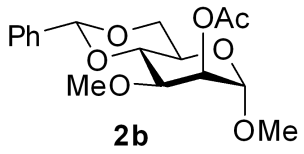

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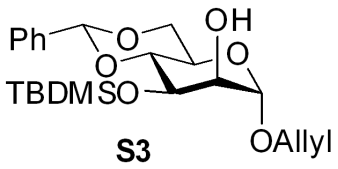

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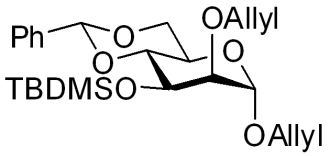

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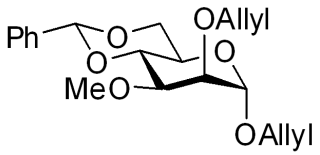

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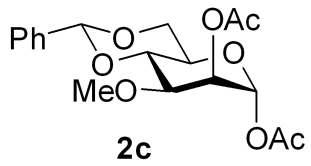

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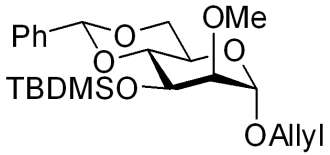

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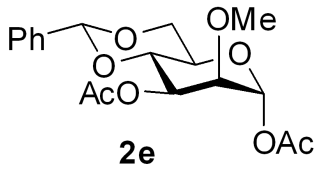

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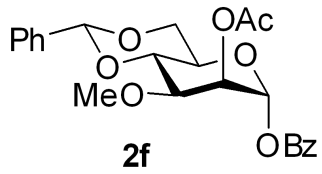

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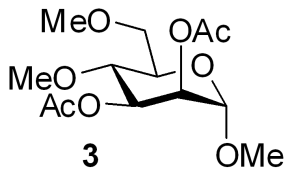

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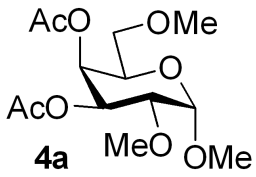

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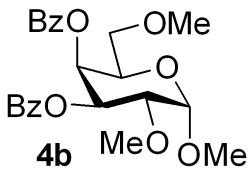

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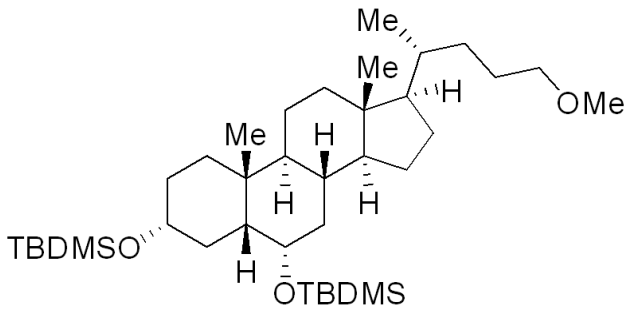

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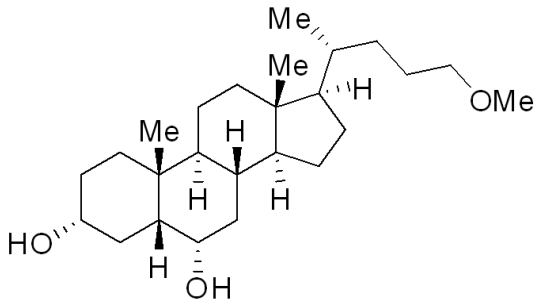

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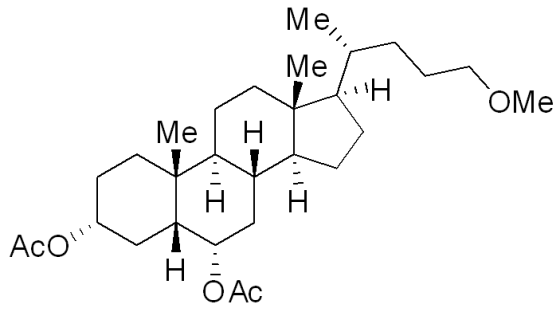

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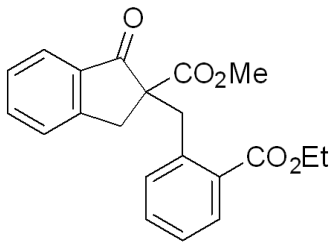

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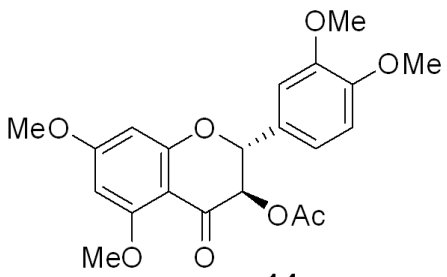

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