Stereoselective Syntheses of Diastereomers of Antitumor Natural Product Pericosine A from (–)-Quinic Acid

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This paper is dedicated to Professor Hiroshi Shimizu on the occasion of his retirement from Gifu Pharmaceutical University.

Abstract: The stereoselective syntheses of diastereomers of antitumor natural pericosine A from (–)-quinic acid were achieved. One of the diastereomers, methyl (3*R*,4*S*,5*S*,6*R*)-6-chloro-3,4,5-trihydroxycyclohex-1-enecarboxylate, possesses the reported structure of pericosine A. The reported relative configuration of natural pericosine A was shown to be incorrect.

Key words: stereoselective synthesis, marine natural product, pericosine A, diastereomer

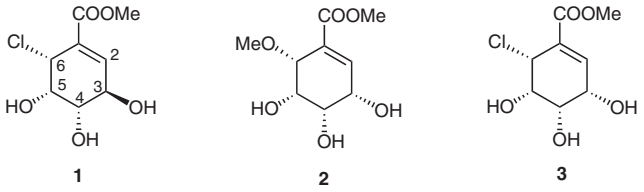
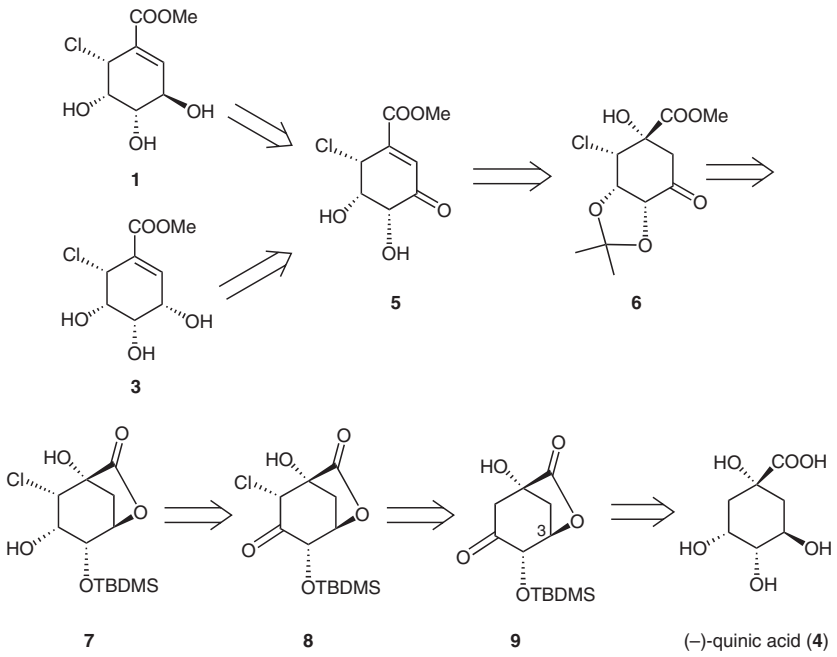


Figure 1

The search for bioactive marine natural products has been pursued intensively over the past two decades.[1,2] A new trend has emerged that involves the study of the metabolites of microorganisms from marine sources.[3,4] In the course of such studies, unique multifunctionalized C_7-cyclohexenoids, designated as pericosines A and B, were isolated in 1977 as metabolites of *Periconia byssoides* OUPS-N133 originally separated from the sea hare, *Aplysia kurodai*.[5] The reported structures **1** and **2** are presented in Figure 1. These compounds showed significant *in vitro* cytotoxicity against P388 lymphocytic leukemia cells (ED_{50} 0.12 and 4.0 μg/mL, respectively).

Pericosines have the structural character of carbasugars, and have been reported to exhibit antiviral, antifungal, and antitumor activities.[6] We are interested in the syntheses of natural pericosines and related compounds.[7,8]

In 1998, Donohoe and co-workers achieved the total synthesis of **2** and established its absolute configuration.[9] In 2005, Ruano and co-workers reported their synthetic efforts to produce **2** using asymmetric Diels–Alder reaction, but their total synthesis could not be completed.[10]



Scheme 1 Retrosynthetic strategy for **1** and **3** from (–)-quinic acid

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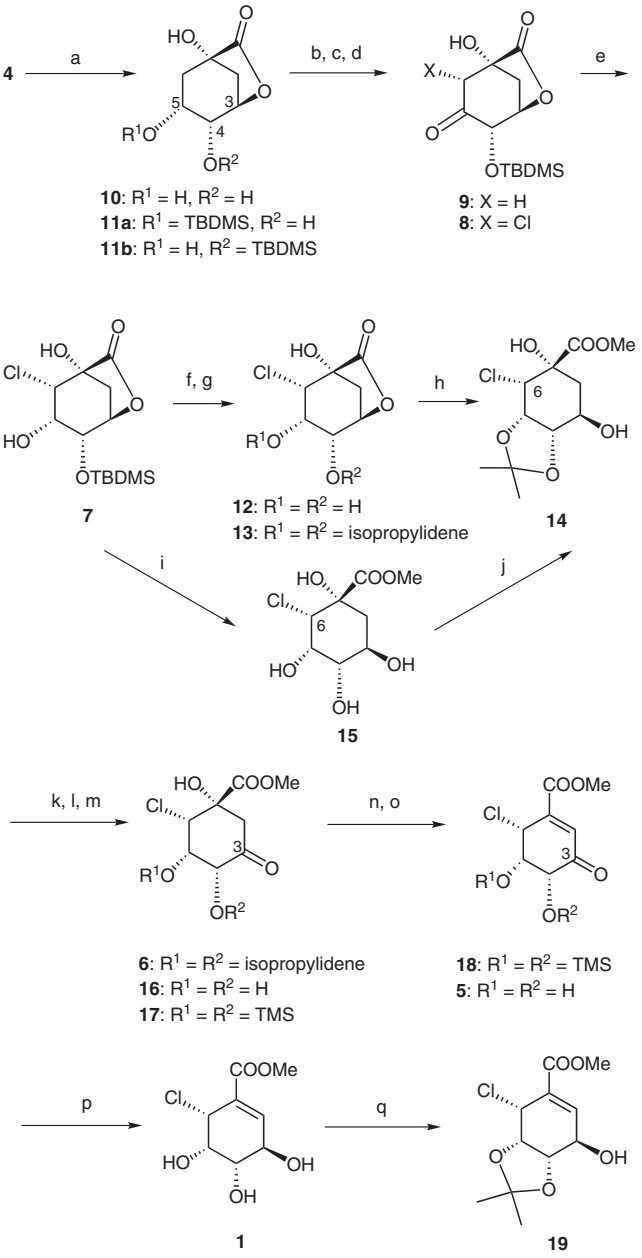
In spite of the significant *in vivo* antitumor activity of pericosine A against murine P388 leukemia cells (the T/C value was estimated to be 122%),[5] there has been no report of its absolute configuration. Thus, we have attempted the stereoselective total synthesis of **1** in order to elucidate the absolute configuration of pericosine A as a promising seed compound for cancer drug candidates. We have already stated that **1** is an incorrect structure for pericosine A in a preliminary report.[11] We describe herein the full details of the stereoselective syntheses of diastereomers **1** and **3** of pericosine A from (–)-quinic acid (**4**),[12,13] together with the results of ^{1}H and 2D NMR analysis of the intermediates and the products. Improvements of chemical yields were also examined.

Our synthetic strategy is summarized in Scheme 1. Both synthetic target molecules **1** and **3** possessing a different configuration at C3 could be prepared from common intermediate enone **5** by independent stereoselective reduction of the C3 ketone in **5**. Enone **5** was prepared by dehydration of β-hydroxy ketone **6**, which was obtained through the lactone ring opening of diol **7**. Diol **7** was obtained by stereoselective reduction of α-chloro ketone **8**. α-Chloro ketone **8** was prepared by the stereoselective chlorination of known ketone **9** derived from commercially available **4**.

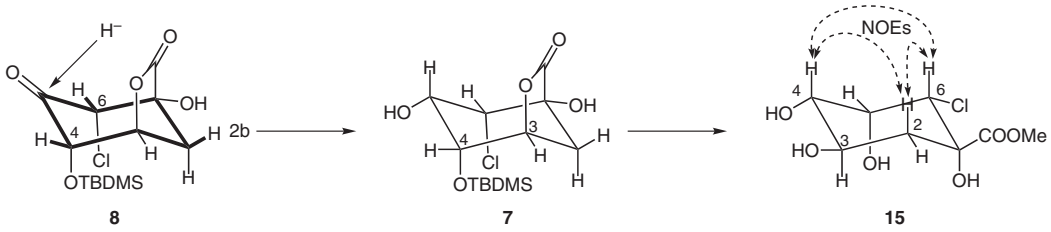
The synthesis of **1** was achieved as shown in Scheme 2. Using a literature procedure,[14] **4** was converted into lactone **10**, which was silylated to give a mixture of 4-silyl ether **11a** and 5-silyl ether **11b**. These were separated and the pure *tert*-butyldimethylsilyl ether **11b** was oxidized with Dess–Martin periodinane to give known lactone **9** in 95% yield. The trimethylsilyl enol ether derived from **9** was chlorinated with *N*-chlorosuccinimide in *N*,*N*-dimethylformamide with excellent selectivity to give the desired α-chloro ketone **8** in 45% yield in two steps.

The stereochemistry at C6 was deduced from the low-field shift (δ = 3.29) of H2α in the ^{1}H NMR spectrum of **8** compared with that of **9** (δ = 2.86). This stereoselectivity was the same as for bromination or fluorination described in the literature[14,15] showing that the bromo ketone assumed a chair conformation with 6α-axial-bromo atom, causing the low-field shift of H2α_{ax}. Long-range couplings H6/H2$_{eq}$ (J = 2.5 Hz), H4/H2$_{eq}$ (J = 1.1 Hz), and H4/H6 (J = 1.4 Hz) in **8** also supported its chair conformation (Scheme 3). The proximity of the chlorinating agent to the silyl enol ether from the opposite side of the hindered lactone bridge was surmised to cause this stereoselectivity.

The following reduction of **8** with sodium borohydride gave single diol **7** in 68% yield with the desired stereochemistry that was proved completely, as described later. The chemical yield of **7** was improved by sequential reactions from 45% in two steps to an overall yield of 57% in three steps from ketone **9**. The stereoselectivity observed in this reduction was explained with the Felkin–Anh model.[16,17] In this case, the lactone bridge should play a role in fixing the conformation of the cyclohexane ring. Electronegative C4–O (α_{ax}) and C6–Cl (α_{ax}) should stabilize the Felkin–Anh transition state for the β-side hydride attack to C5 carbonyl (Scheme 3), despite the presence of a lactone bridge in the molecule. An alternative plausible explanation was steric repulsion effected by neighboring bulky substituents at C4 or C6.



Scheme 2 *Reagents and conditions*: (a) Ref. 14; (b) Dess–Martin periodinane, CH_2Cl_2 (95%); (c) TMSOTf, Et_3N, toluene, reflux; (d) NCS, DMF (33% in 2 steps from **9**); (e) $NaBH_4$, MeOH (68%), (57% in 3 steps from **9**); (f) Bu_4NF, THF (67%); (g) 2,2-dimethoxypropane, PPTS, acetone, reflux (30%); (h) NaOMe, MeOH (90%); (i) TFA, MeOH, 60 °C (38%); (j) 2,2-dimethoxypropane, cat. TsOH, CH_2Cl_2 (65%); (k) Dess–Martin periodinane, CH_2Cl_2 (78%); (l) TFA, MeOH (70%); (m) TMSCl, Et_3N (71%); (n) Martin's sulfurane dehydrating agent, CH_2Cl_2 (65%); (o) TFA, MeOH (quant.); (p) $Bu_4NBH(OAc)_3$, AcOH–AcCN (64%); (q) 2,2-dimethoxypropane, cat. TsOH, acetone (72%).



Scheme 3

Diol **7** was treated with tetrabutylammonium fluoride to afford triol **12** in 67% yield, which was then transformed into acetonide **13** in 30% yield. The lactone ring of **13** was opened with sodium methoxide to afford diol **14** in 90% yield.

Secondary alcohol **14** was oxidized with Dess–Martin periodinane[18] to give hydroxy ketone **6** in 78% yield. The NOESY cross peaks H4/H2β, H6/H2β, and H4/H6 were observed in **6**.

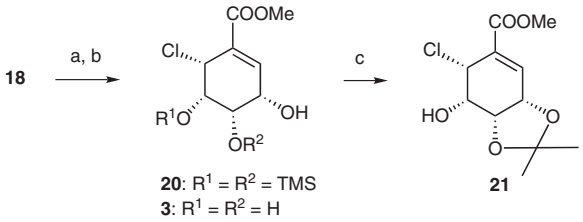
Because of the low chemical yields of these steps, an alternative route was examined. Diol **7** was converted into tetraol **15** in 38% yield with trifluoroacetic acid in methanol under reflux with recovery of dechlorinated methyl quinate in 30% yield. Compound **15** was confirmed to have the chair conformation based on analyses of the coupling constants of ^{1}H NMR and NOESY cross peaks H4/H2β, H6/H2β, and H4/H6 (Scheme 3). This experiment proved the stereochemistry at C6 in **8** or C5 in **7**. Treatment of **15** with 2,2-dimethoxypropane and catalytic 4-toluenesulfonic acid in dichloromethane under reflux gave **14** in 65% yield.

However, **6** was not dehydrated with various kinds of dehydrating agents. Then, the isopropylidene moiety of **6** was removed by reacting with trifluoroacetic acid to give triol **16** in 70% yield, which was then protected as the bis(trimethylsilyl ether) to give reactive hydroxy ketone **17** in 71% yield. The HMBC cross peak H2α/C6 in **16** confirmed the assignment of the NMR signals. Observing NOESY cross peaks between H4 and H6 in **6**, **16**, and **17** implied that no epimerization had occurred during the preparation of these compounds.

Dehydration of **17** to give α,β-unsaturated ketone **18** was carried out with Martin's sulfurane dehydrating agent (bis[α,α-bis(trifluoromethyl)benzyloxy]diphenylsulfur)[19] in 65% yield. Dehydration with thionyl chloride and triethylamine afforded **18** in 30% yield together with the deprotected dihydroxy α,β-unsaturated ketone **5** in 16% yield. The HMBC cross peaks H6/C1, H5/C1, H2/C1, H4/C3, and H5/C3 in **18** led the assignment of the NMR signals. The cross peak between H4 and H6 in the NOESY spectra of **18** implied that H4 and H6 had the *cis* configuration.

Enone **18** was treated with trifluoroacetic acid and methanol to give **5** quantitatively, and **5** was reduced with tetrabutylammonium triacetoxyborohydride[20] in a stereoselective manner to give **1**[5] having the desired stereostructure in 64% yield. Proof of the stereochemistry will be described later.

However, the NMR data of **1** and acetonide **19** did not agree with those of natural pericosine A and those of its acetonide described in the literature.[5] Furthermore, the isopropylidene bridge in **19** derived from **1** was located between C4 and C5, whereas that of the acetonide of pericosine A was between C3 and C4.[5] This was supported by NOESY cross peaks between one of two methyl groups and H4 or H5 of **19**. These NOESY cross peaks in **19** and the NOESY cross peaks H4/H6 in **18** described above also denied the possibility of epimerization at C4 and C6 during the conversion from **17** into **18**.



Scheme 4 *Reagents and conditions*: (a) $NaBH_4$, MeOH; (b) TFA, CH_2Cl_2 (quant. in 2 steps); (c) 2,2-dimethoxypropane, cat. TsOH (55%).

The stereochemistry at C3 in **1** was proved by the following experiments. Common intermediate **18** was reduced with sodium borohydride to afford allyl alcohol **20** as a single product. Then, **20** was deprotected to afford our second target diastereomer **3** quantitatively in two steps (Scheme 4). Product **3** was transformed into 3,4-*O*-acetonide **21** in 55% isolated yield. NOESY cross peaks between one of two methyl groups of the isopropylidene moiety and H3 or H4 implied that the newly generated hydroxy group at C3 and the neighboring hydroxy group at C4 had the *cis* configuration in **21**. Furthermore a NOESY cross peak between H3 and H5 in **3** implied that H3 and H5 had the *cis* configuration. Therefore, the stereochemistry of synthesized **3** was confirmed (Scheme 4). The spectral data of **3** were different from those of **1** or natural pericosine A. Based on these experiments, synthesized **1** must have an α-hydroxy group at C3. All the experimental results described above guaranteed correctness of the stereostructure of synthesized **1**.

Through this study, we have shown that **1** was the incorrect stereostructure of pericosine A.

In conclusion, the stereoselective syntheses of diastereomers **1** and **3** of pericosine A from (–)-quinic acid were achieved. Detailed NMR analysis of the intermediates or the products confirmed that the stereostructures of **1** and **3** were methyl (3*R*,4*S*,5*S*,6*R*)- and (3*S*,4*S*,5*S*,6*R*)-6-chloro-3,4,5-trihydroxycyclohex-1-enecarboxylate, respectively. This study confirmed that the structure of antitumor natural pericosine A was not **1** or its diastereomer **3**. The results of this study have motivated us to conduct the total synthesis of real pericosine A assigned to (3*S*,4*S*,5*S*,6*S*)-6-chloro-3,4,5-trihydroxycyclohex-1-enecarboxylate as our next work.[21,22]

IR spectra were obtained with a Perkin Elmer FT-IR spectrometer 1720X. EIMS was determined with a Hitachi 4000H mass spectrometer. NMR spectra were recorded at 27 °C on Varian Unity Inova-500, Gemini-2000, and Mercury-300 spectrometers in $CDCl_3$ with TMS as internal reference; for comparative purposes, the assignments are always given according to the numbering given in Scheme 2. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Liquid column chromatography was conducted over silica gel (Nacalai Tesque, silica gel 60, mesh 70–230 or 230–400). Analytical TLC was performed on precoated Merck aluminum sheets (DC-Alufolien Kieselgel 60 F_{254}), and compounds were detected by spraying an EtOH soln of phosphomolybdic acid followed by heating. Anhyd THF was distilled over sodium benzophenone ketyl under argon atmosphere.

(1*S*,3*R*,4*S*)-4-(*tert*-Butyldimethylsiloxy)-1-hydroxy-5-oxocyclohexane-1,3-carbolactone (9)

To a suspension of Dess–Martin periodinane (3.74 g, 8.82 mmol) in CH_2Cl_2 (10 mL) was added **11b** (1.27 g, 4.41 mmol) in CH_2Cl_2 (2 mL) at r.t. After stirring for 2 h, the mixture was diluted with *t*-BuOMe and treated with aq $Na_2S_2O_3$ and aq $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to give a crude residue that was recrystallized to afford **9** (1.20 g, 95%).[14]

(1*S*,3*R*,4*S*,6*R*)-4-*tert*-(Butyldimethylsiloxy)-6-chloro-1-hydroxy-5-oxocyclohexane-1,3-carbolactone (8)

To a toluene soln (6 mL) of ketone **9** (284 mg, 1.00 mmol) were added successively Et_3N (578 μL, 4.00 mmol) and TMSOTf (609 μL, 3.4 mmol) and the resulting soln was refluxed for 8 h with stirring under argon. The mixture was diluted with hexane mixed with aq $NaHCO_3$ and extracted. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the crude silyl enol ether. To the soln of silyl enol ether in DMF (5 mL) was added NCS (133 mg, 1.00 mmol) and the mixture was stirred at r.t. for 50 h under argon. The resulting mixture was extracted with *n*-hexane, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the crude chloro ketone that was purified by recrystallization to afford **8** (107 mg, 33%) as colorless crystals (Et_2O); mp 67–70 °C.

$[\alpha]_D^{26}$ –11.0 (*c* 1.7, MeOH).

IR (KBr): 3521 (OH), 1820 (C=O), 1739 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.13 (s, 3 H, SiMe), 0.16 (s, 3 H, SiMe), 0.90 (s, 9 H, *t*-Bu), 2.51 (dddd, *J* = 12.6, 6.3, 2.5, 1.1 Hz, 1 H, H2β), 3.29 (d, *J* = 12.6 Hz, 1 H, H2α), 4.13 (ddd, *J* = 4.1, 1.4, 1.1 Hz, 1 H, H4), 4.17 (dd, *J* = 2.5, 1.4 Hz, 1 H, H6), 4.74 (dd, *J* = 6.3, 4.1 Hz, 1 H, H3).

^{13}C NMR ($CDCl_3$): δ = –5.2 (q), –5.0 (q), 18.1 (s), 25.5 (3 q), 31.4 (t), 62.1 (d), 71.1 (d), 74.6 (d), 74.9 (d), 173.6 (s), 197.7 (s).

MS (CI): *m*/*z* = 321 $[M + H]^+$.

HRMS: *m*/*z* $[M - t\text{-Bu}]^+$ calcd for $C_9H_{12}O_5SiCl$: 263.0142; found: 263.0129.

Anal. calcd for $C_{13}H_{21}ClO_5Si \cdot 0.25\ H_2O$: C, 47.96; H, 6.73; found; C, 47.89; H, 6.74.

(1*S*,3*R*,4*R*,5*S*,6*S*)-4-(*tert*-Butyldimethylsiloxy)-6-chloro-1,5-dihydroxycyclohexane-1,3-carbolactone (7)

Method A: A soln of chloro ketone **8** (32.1 mg, 0.10 mmol) in MeOH (1 mL) was added dropwise to a suspension of $NaBH_4$ (3.8 mg, 0.10 mmol) in MeOH (2 mL) at –15 °C. The mixture was stirred for 30 min, quenched with H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give crude diol that was purified by chromatography (2% MeOH–CH_2Cl_2) to afford **7** (22 mg, 68%).

Method B: Crude chloro ketone **9**, which was obtained by the same procedure as that described above on the same scale (1.0 mmol of starting material **11b**), was dissolved in THF (3 mL) and the mixture was added to a suspension of $NaBH_4$ (37.8 mg, 1 mmol) in MeOH (3 mL) at –15 °C. The mixture was treated in the same manner to afford **7** (184.0 mg, 57% from starting ketone) as colorless crystals; mp 128–131 °C (CH_2Cl_2).

$[\alpha]_D^{26}$ –44.5 (*c* 0.45, MeOH).

IR (KBr): 3437 (OH), 1807 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.11 (s, 3 H, SiMe), 0.16 (s, 3 H, SiMe), 0.94 (s, 9 H, *t*-Bu), 2.24 (ddd, *J* = 12.4, 6.0, 1.4 Hz, 1 H, H2β), 2.75 (d, *J* = 12.4 Hz, 1 H, 5-OH), 3.10 (d, *J* = 12.4 Hz, 1 H, H2α), 3.94 (ddd, *J* = 12.4, 5.2, 4.4 Hz, 1 H, H5), 4.12 (dd, *J* = 4.9, 4.4 Hz, 1 H, H4), 4.35 (dd, *J* = 5.2, 1.4 Hz, 1 H, H6), 4.67 (dd, *J* = 6.0, 4.9 Hz, 1 H, H3).

^{13}C NMR ($CDCl_3$): δ = –4.9 (q), –4.6 (q), 17.9 (s), 25.6 (3 q), 32.0 (t), 65.7 (d), 66.7 (d), 67.3 (d),75.5 (s), 76.0 (d), 174.3 (s).

HRSIMS: *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{24}ClO_5Si$: 323.1080; found: 323.1084.

(1*S*,3*R*,4*R*,5*S*,6*S*)-6-Chloro-1,4,5-trihydroxycyclohexane-1,3-carbolactone (12)

To a soln of **7** (257 mg, 0.80 mmol) in THF (3 mL) was added 1.0 M Bu_4NF in THF (0.8 mL, 0.80 mmol,) under argon. The mixture was stirred at r.t. for 3 h, and $TsOH \cdot H_2O$ (152 mg, 0.80 mmol) was added. The mixture was stirred for a further 30 min and concentrated directly under reduced pressure to give a residue that was purified by column chromatography (silica gel, gradient, EtOAc–hexane, 1:1 to EtOAc) to afford **12** (111.1 mg, 67%) as colorless crystals; mp 111–115 °C (EtOAc–hexane).

$[\alpha]_D^{26}$ –57.5 (*c* 0.58, MeOH).

IR (KBr): 3403 (OH), 1785 (C=O) cm^{-1}.

^1H NMR (CD_3OD): δ = 2.14 (ddd, *J* = 12.1, 6.0, 2.2 Hz, 1 H, H2β), 3.03 (d, *J* = 12.1 Hz, 1 H, H2α), 3.86 (br t, *J* = 4.7 Hz, 1 H, H4), 4.04 (t, *J* = 4.7 Hz, 1 H, H5), 4.29 (ddd, *J* = 4.7, 2.2, 1.1 Hz, 1 H, H6), 4.71 (dd, *J* = 6.0, 4.7 Hz, 1 H, H3).

^{13}C NMR (CD_3OD): δ = 32.8 (t), 67.0 (d), 67.2 (d), 67.6 (d), 76.7 (s), 77.2 (d), 177.1 (s).

HRMS (CI): *m*/*z* $[M + H]^+$ calcd for $C_7H_{10}ClO_5$: 209.0216; found: 209.0217.

(1*S*,3*R*,4*R*,5*S*,6*S*)-6-Chloro-1-hydroxy-4,5-(isopropylidenedioxy)cyclohexane-1,3-carbolactone (13)

A mixture of **12** (73.8 mg, 0.36 mmol), PPTS (8.9 mg, 0.04 mmol), and 2,2-dimethoxypropane (1 mL) in acetone (5 mL) was refluxed for 2 h, cooled to r.t., and concentrated to give a crude residue that

was purified by column chromatography (silica gel, EtOAc–*n*-hexane, 1:1) to afford **13** (26.0 mg, 30%) as colorless crystals (CH_2Cl_2); mp 72–75 °C.

$[\alpha]_D^{26}$ +4.4 (*c* 0.68, MeOH).

IR (KBr): 3437 (OH), 1768 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.41 (s, 3 H, acetonide-Me), 1.67 (s, 3 H, acetonide-Me), 2.36 (dd, *J* = 12.1, 5.2 Hz, 1 H, H2β), 2.95 (d, *J* = 12.1 Hz, 1 H, H2α), 4.39 (m, 1 H), 4.48 (m, 2 H), 4.99 (m, 1 H).

^{13}C NMR ($CDCl_3$): δ = 25.4 (q), 25.5 (q), 31.9 (t), 63.3 (d), 72.0 (d), 72.5 (d), 74.4 (s), 75.1 (s), 113.1 (s), 174.1 (s).

MS (CI): *m*/*z* = 249 $[M + H]^+$.

HRMS: *m*/*z* $[M - CH_3]^+$ calcd for $C_9H_{10}ClO_5$: 233.0216; found: 233.0221.

Methyl (1*S*,3*R*,4*S*,5*S*,6*S*)-6-Chloro-1,3-dihydroxy-4,5-(isopropylidenedioxy)cyclohexanecarboxylate (14)

To a soln of **13** (24.0 mg, 0.10 mmol) in MeOH (1 mL) was added NaOMe (7.0 mg, 0.13 mmol). The mixture was stirred at r.t. for 1 h, after which a drop of AcOH was added and stirring was continued for a further 15 min. The mixture was concentrated directly to give a crude residue that was purified by column chromatography (silica gel, 3% MeOH–CH_2Cl_2) to afford **14** (24.4 mg, 90%) as a colorless oil.

$[\alpha]_D^{26}$ +9.5 (*c* 1.71, MeOH).

IR (liquid film): 3474 (OH), 1742 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.41 (s, 3 H, acetonide-Me), 1.59 (s, 3 H, acetonide-Me), 1.94 (dd, *J* = 14.0, 10.7 Hz, 1 H, H2β), 2.24 (dd, *J* = 14.0, 4.4 Hz, 1 H, H2α), 2.55 (br s, 1 H OH), 3.58 (s, 1 H, OH), 3.86 (s, 3 H, COOMe), 4.06 (dd, *J* = 6.9, 5.2 Hz, 1 H, H4), 4.17 (ddd, *J* = 10.7, 6.9, 4.4 Hz, 1 H, H3), 4.53 (dd, *J* = 5.2, 4.1 Hz, 1 H, H5), 4.60 (d, *J* = 4.1 Hz, 1 H, H6).

^{13}C NMR ($CDCl_3$): δ = 25.8 (q), 28.2 (q), 38.6 (t), 53.7 (q), 58.8 (d), 67.1 (d), 76.67 (d), 76.71 (s), 80.4 (d), 110.3 (s), 173.2 (s).

MS (CI): *m*/*z* = 281 $[M + H]^+$.

HRMS: *m*/*z* $[M - CH_3]^+$ calcd for $C_{10}H_{14}ClO_6$: 265.0478; found: 265.0483.

Methyl (1*S*,3*R*,4*S*,5*S*,6*S*)-6-Chloro-1,3,4,5-tetrahydroxycyclohexanecarboxylate (15)

Compound **7** (476 mg, 1.47 mmol) was dissolved in MeOH (4 mL) and TFA (2 mL, excess) and the reaction flask was heated overnight at 60 °C. After cooling, the solvent was removed under reduced pressure to give a residue that was purified by column chromatography (silica gel, 2% then 5% then 10% MeOH–CH_2Cl_2 gradient) to afford pure **15** (129 mg, 38%) as colorless crystals; mp 157–160 °C; together with methyl quinate (87.6 mg, 30%).

$[\alpha]_D^{26}$ –20.9 (*c* 0.49, MeOH).

IR (liquid film): 3441 (OH), 1748 (C=O) cm^{-1}.

^1H NMR (CD_3OD): δ = 1.81 (dd, *J* = 13.5, 11.7 Hz, 1 H, H2β), 2.19 (dd, *J* = 13.5, 5.0 Hz, 1 H, H2α), 3.45 (dd, *J* = 9.6, 3.0 Hz, 1 H, H4), 3.78 (s, 3 H, COOMe), 3.98 (ddd, *J* = 11.7, 9.6, 5.0 Hz, 1 H, H3), 4.10 (dd, *J* = 3.0, 2.8 Hz, 1 H, H5), 4.49 (d, *J* = 2.8 Hz, 1 H, H6).

^{13}C NMR (CD_3OD): δ = 42.4 (t), 53.4 (q), 62.1 (d), 66.7 (d), 76. 7 (d), 76.7 (2 d), 80.6 (s), 173.8 (s).

HRMS: *m*/*z* $[M + H]^+$ calcd for $C_8H_{14}ClO_6$: 241.0478; found: 241.0482.

Anal. Calcd for $C_8H_{13}O_6Cl$: C, 39.30; H, 5.45; found: C, 39.42; H, 5.46.

Methyl (1*S*,2*S*,3*S*,4*S*,5*R*)-2-Chloro-1,5-dihydroxy-3,4-(isopropylidenedioxy)cyclohexanecarboxylate (14) from 15

2,2-Dimethoxypropane (1 mL, excess) and a catalytic amount of $TsOH{\cdot}H_2O$ (2.0 mg) were added to a CH_2Cl_2 soln (5 mL) of **15** (22.0 mg, 0.09 mmol). The mixture was stirred under reflux with 3A molecular sieves for 2 h. After removing the volatile components under reduced pressure, CH_2Cl_2 was added to the residue. The supernatant that was separated from the residue was concentrated under reduced pressure to give almost pure **14** (16.8 mg, 65%).

Methyl (1*S*,4*S*,5*R*,6*S*)-6-Chloro-1-hydroxy-4,5-(isopropylidenedioxy)-3-oxocyclohexanecarboxylate (6)

To a suspension of Dess–Martin periodinane (579 mg, 1.35 mmol) in CH_2Cl_2 (5 mL) was added **14** (190 mg, 0.68 mmol) in CH_2Cl_2 (2 mL) at r.t. After stirring for 2 h, the mixture was diluted with *t*-BuOMe and treated with aq $Na_2S_2O_3$ and aq $NaHCO_3$. The organic layer was washed with brine, dried ($MgSO_4$), and filtered, and the solvent was removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 1% MeOH–CH_2Cl_2) to afford **6** (146.2 mg, 78%) as colorless crystals (Et_2O–hexane); mp 115–118 °C.

$[\alpha]_D^{26}$ +33.9 (*c* 0.45, MeOH).

IR (liquid film): 3275 (OH), 1760 (C=O), 1744 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.44 (s, 3 H, acetonide-Me), 1.50 (s, 3 H, acetonide-Me), 2.85 (d, *J* = 14.4 Hz, 1 H, H2α), 3.00 (d, *J* = 14.4 Hz, 1 H, H2β), 3.89 (s, 3 H, COOMe), 4.52 (d, *J* = 5.5 Hz, 1 H, H4), 4.81 (dd, *J* = 5.5, 3.9 Hz, 1 H, H5), 4.91 (d, *J* = 3.9 Hz, 1 H, H6).

^{13}C NMR ($CDCl_3$): δ = 26.0 (q), 27.0 (q), 47.4 (t), 53.8 (q), 57. 8 (d), 78.5 (d), 78.8 (d), 79.3 (s), 111.9 (s), 171.0 (s), 201.4 (s).

MS (EI): *m*/*z* = 263 $[M - CH_3]^+$.

HRMS: *m*/*z* $[M - CH_3]^+$ calcd for $C_{10}H_{12}ClO_6$: 263.0321; found: 263.0326.

Methyl (1*S*,4*S*,5*R*,6*S*)-6-Chloro-1,4,5-trihydroxy-3-oxocyclohexanecarboxylate (16)

Compound **6** (16.1 mg, 0.058 mmol) was dissolved in MeOH (3 mL) and TFA (1 mL, excess) was added with stirring at r.t. After 1 h, the solvent was removed from the mixture under reduced pressure to afford a residue that was purified by column chromatography (silica gel, 5% MeOH–CH_2Cl_2) to afford **16** (10.1 mg, 70%) as a colorless oil.

$[\alpha]_D^{24}$ +16.7 (*c* 0.36, MeOH).

IR (liquid film): 3470 (OH), 1736 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 2.91 (d, *J* = 14.1 Hz, 1 H, H2β), 3.07 (dd, *J* = 14.1, 0.9 Hz, 1 H, H2α), 3.92 (s, 3 H, COOMe), 4.42 (dd, *J* = 3.8, 0.9 Hz, 1 H, H4), 4.56 (dd, *J* = 3.8, 2.7 Hz, 1 H, H5), 4.80 (d, *J* = 2.7 Hz, 1 H, H6).

^{13}C NMR ($CDCl_3$): δ = 47.4 (t), 54.1 (q), 59.1 (d), 76.8 (d), 77.3 (d), 81.0 (s), 170.7 (s), 203.0 (s).

HRMS: *m*/*z* $[M - H_2O]^+$ calcd for $C_8H_9ClO_5$: 220.0138; found: 220.0145.

Methyl (1*S*,4*S*,5*R*,6*S*)-6-Chloro-1-hydroxy-4,5-bis(trimethylsiloxy)-3-oxocyclohexanecarboxylate (17)

To soln of **16** (6.2 mg, 0.026 mmol) in CH_2Cl_2 (2 mL) were added Et_3N (14.5 μL, 0.10 mmol) and TMSCl (9.9 μL, 0.09 mmol,). The mixture was stirred overnight at r.t., treated with sat. aq NH_4Cl, and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 1% MeOH–CH_2Cl_2) to afford **17** (7.1 mg, 71%) as colorless crystals (CH_3Cl); mp <40 °C.

$[\alpha]_D^{22}$ +23.4 (*c* 0.57, MeOH).

IR (KBr): 3497 (OH), 1737 (C=O), 1732 (C=O) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.16 (s, 9 H, 4-OTMS), 0.18 (s, 9 H, 5-OTMS), 2.89 (d, *J* = 14.9 Hz, 1 H, H2β), 2.97 (dd, *J* = 14.9, 0.9 Hz, 1 H, H2α), 3.85 (s, 3 H, COOMe), 4.38 (dd, *J* = 3.0, 0.9 Hz, 1 H, H4), 4.50 (dd, *J* = 3.0, 2.1 Hz, 1 H, H5), 4.78 (d, *J* = 2.1 Hz, 1 H, H6), 4.89 (s, 1 H, OH).

^{13}C NMR ($CDCl_3$): δ = 0.05 (q), 0.1 (q), 49.2 (t), 53.4 (q), 59.7 (d), 77.8 (d), 81.1 (s), 81.2 (d), 171.1 (s), 201. 3 (s).

HRMS: *m/z* $[M]^+$ calcd for $C_{14}H_{27}ClO_6Si_2$: 382.1021; found: 382.1027.

Methyl (4*R*,5*S*,6*R*)-6-Chloro-4,5-bis(trimethylsiloxy)-3-oxocyclohex-1-enecarboxylate (18)

Method A: To a CH_2Cl_2 soln (5 mL) of **17** (79.5 mg, 0.21 mmol) were added Me_3N (228 μL, 2.10 mmol) and $SOCl_2$ (153 μL, 2.10 mmol) at –10 °C. After stirring at 0 °C for 3 h, the mixture was treated with sat. aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 1% MeOH–CH_2Cl_2) to afford **18** (22.6 mg, 30%) and desilylated **16** (7.3 mg, 16%).

Method B: To a CH_2Cl_2 soln (1 mL) of **17** (7.9 mg, 0.021 mmol) was added 1 M Martin's sulfurane dehydrating agent in CH_2Cl_2 (0.93 mL, 0.93 mmol) at 0 °C, and the mixture was stirred for 3 h. The mixture was treated in the same manner as method A to afford **18** (4.9 mg, 65%) as colorless crystals (CH_2Cl_2); mp 88–90 °C.

$[\alpha]_D^{22}$ +34.4 (*c* 0.87, MeOH).

IR (KBr): 1732 (C=O), 1711 (C=O), 1508 (C=C) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 0.12 (s, 9 H, OTMS), 0.17 (s, 9 H, OTMS), 3.86 (s, 3 H, COOMe), 4.27 (d, *J* = 2.3 Hz, 1 H, H4), 4.37 (dd, *J* = 3.4, 2.3 Hz, 1 H, H5), 5.12 (dd, *J* = 3.4, 2.3 Hz, 1 H, H6), 6.53 (d, *J* = 2.3 Hz, 1 H, H2).

^{13}C NMR ($CDCl_3$): δ = 0.15 (q), 0.3 (q), 52.7 (q), 56.5 (d), 76.5 (d), 77.9 (d), 130.6 (d), 143.7 (s), 165.5 (s), 195.8 (s).

HRMS: *m/z* $[M]^+$ calcd for $C_{14}H_{25}{}^{35}ClO_5Si_2$: 364.0927; found: 364.0930.

Methyl (4*R*,5*S*,6*R*)-6-Chloro-4,5-dihydroxy-3-oxocyclohex-1-enecarboxylate (5)

Compound **18** (27.3 mg, 0.075 mmol) was dissolved in MeOH (1 mL) and TFA (0.5 mL, excess) was added with stirring at r.t. After 15 min, the solvent was removed from the mixture under reduced pressure to afford a residue that was purified by column chromatography (silica gel, 2% MeOH–CH_2Cl_2) to afford **5** (16.7 mg, 100%) as colorless crystals (CH_2Cl_2); mp 97–100 °C.

$[\alpha]_D^{22}$ undetermined (almost 0).

IR (KBr): 3501 (OH), 1725 (C=O), 1708 (C=O), 1625 (C=C) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 3.90 (s, 3 H, COOMe), 4.37 (d, *J* = 2.5 Hz, 1 H, H4), 4.60 (dd, *J* = 3.4, 2.5 Hz, 1 H, H5), 5.27 (dd, *J* = 3.4, 2.3 Hz, 1 H, H6), 6.72 (d, *J* = 2.3 Hz, 1 H, H2).

^{13}C NMR ($CDCl_3$): δ = 53.0 (q), 56.3 (d), 73.2 (d), 76.0 (d), 129.6 (d), 144.7 (s), 164.8 (s), 196.4 (s).

MS (CI): *m/z* = 221 $[M + H]^+$.

HRMS: *m/z* $[M - H_2O]^+$ calcd for $C_8H_7{}^{35}ClO_4$: 202.0030; found: 202.0031.

Methyl (3*R*,4*S*,5*S*,6*R*)-6-Chloro-3,4,5-trihydroxycyclohex-1-enecarboxylate (1)

To a soln of $Me_4NBH(OAc)_3$ (52.8 mg, 0.20 mmol,) in AcOH–MeCN (1:1, 1 mL) was added dropwise **5** (5.8 mg, 0.026 mmol) in MeCN (0.5 mL) at –20 °C. After stirring for 30 min, the mixture was treated with 1.0 M sodium potassium tartrate and sat. aq $NaHCO_3$, and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 5% MeOH–CH_2Cl_2) to afford **1** (3.7 mg, 64%) as an oil.

$[\alpha]_D^{30}$ –71.1 (*c* 0.09, EtOH).

IR (liquid film): 3383 (OH), 1721 (C=O), 1652 (C=C) cm^{-1}.

^1H NMR (acetone-d_6): δ = 3.72 (dd, *J* = 5.9, 2.3 Hz, 1 H, H4), 3.76 (s, 3 H, COOMe), 4.16 (dd, *J* = 4.1, 2.3 Hz, 1 H, H5), 4.60 (m, 1 H, H3), 5.07 (dt, *J* = 4.1, 1.4 Hz, 1 H, H6), 6.73 (d, *J* = 3.0, 1.4 Hz, 1 H, H2).

^{13}C NMR (acetone-d_6): δ = 52.2 (q), 58.0 (d), 69.6 (d), 70.6 (d), 75. 3 (d), 130.9 (d), 141.6 (s), 166.4 (s).

HRMS: *m/z* $[M]^+$ calcd for $C_8H_{12}{}^{35}ClO_5$: 223.0372; found: 223.0360.

Methyl (3*R*,4*S*,5*S*,6*R*)-6-Chloro-3-hydroxy-4,5-(isopropylidenedioxy)cyclohex-1-enecarboxylate (19)

To a soln of **1** (2.0 mg, 0.009 mmol) in acetone (0.5 mL) were added 2,2-dimethoxypropane (0.5 mL, excess) and a catalytic amount of $TsOH·H_2O$. After stirring at r.t. for 3 h, volatile components were removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 2% MeOH–CH_2Cl_2) to afford **19** (1.7 mg, 72%) as an oil.

IR (liquid film): 3446 (OH), 1718 (C=O), 1646 (C=C) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.41 (s, 3 H, acetonide-Me), 1.62 (s, 3 H, acetonide-Me), 3.82 (s, 3 H, COOMe), 4.31 (ddd, *J* = 8.7, 5.7, 0.7 Hz, 1 H, H4), 4.36 (dd, *J* = 8.7, 4.8 Hz, 1 H, H5), 4.98 (dd, *J* = 5.7, 2.1 Hz, 1 H, H3), 5.22 (dd, *J* = 4.8, 0.7 Hz, 1 H, H6), 7.14 (ddd, *J* = 2.1, 1.1, 0.7 Hz, 1 H, H2).

HRMS: *m/z* $[M + H]^+$ calcd for $C_{11}H_{16}{}^{35}ClO_5$: 263.0685; found: 263.0695.

Methyl (3*S*,4*S*,5*S*,6*R*)-6-Chloro-3,4,5-trihydroxycyclohex-1-enecarboxylate (3)

To a suspension of $NaBH_4$ (0.74 mg, 0.02 mmol) in MeOH (1.5 mL) was added dropwise **18** (7.7 mg, 0.02 mmol) in MeOH (0.5 mL) at –10 °C. After stirring for 30 min, the mixture was treated with sat. aq NH_4Cl and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure to give almost pure **20** (7.3 mg) as an oil.

^1H NMR ($CDCl_3$): δ = 0.17 [s, 9 H, $Si(CH_3)_3$], 0.18 [s, 9 H, $Si(CH_3)_3$], 3.79 (s, 3 H, COOMe), 4.77 (br d, *J* = 4.2 Hz, 1 H, H6), 6.81 (br d, *J* = 2.6 Hz, 1 H, H2).

Without purification, **20** was dissolved in MeOH (1 mL) and TFA (0.5 mL). After stirring at r.t. for 30 min, the solvent was removed under reduced pressure to afford a residue that was purified by column chromatography (silica gel, 5% MeOH–CH_2Cl_2) to afford **3** (4.8 mg, 100% in 2 steps) as an oil.

$[\alpha]_D^{22}$ +58.3 (*c* 0.01, EtOH).

IR (liquid film): 3373 (OH), 1726 (C=O), 1653 (C=C) cm^{-1}.

^1H NMR (acetone-d_6): δ = 3.78 (s, 3 H, COOMe), 3.89 (ddd, *J* = 8.7, 5.0, 2.3 Hz, 1 H, H5), 4.10 (m, 1 H OH), 4.16 (d, *J* = 9.2 Hz, 1 H, OH), 4.22 (m, 1 H, H3), 4.28 (d, *J* = 8.7 Hz, 1 H, 5-OH), 4.36 (m, 1 H, H4), 5.03 (br d, *J* = 5.0 Hz, 1 H, H6), 6.81 (br d, *J* = 2.5 Hz, 1 H, H2).

^{13}C NMR (acetone-d_6): δ = 52.3 (q), 56.5 (d), 68.8 (d), 67.0 (d), 71.6 (d), 132.0 (d), 142.7 (s), 168.0 (s).

HRMS: *m/z* $[M]^+$ calcd for $C_8H_{12}{}^{35}ClO_5$: 223.0373; found: 223.0379.

Methyl (3*R*,4*R*,5*S*,6*R*)-6-Chloro-5-hydroxy-3,4-(isopropylidenedioxy)cyclohex-1-enecarboxylate (21)

To a soln of **3** (29.8 mg, 0.134 mmol) in acetone (1 mL) were added 2,2-dimethoxypropane (1 mL, excess) and a catalytic amount of $TsOH·H_2O$. After stirring at r.t. for 15 min, volatile components were removed under reduced pressure to give a crude residue that was purified by column chromatography (silica gel, 1% MeOH–CH_2Cl_2) to afford **21** (19.3 mg, 55%) as an oil.

IR (liquid film): 3431 (OH), 1718 (C=O), 1646 (C=C) cm^{-1}.

^1H NMR ($CDCl_3$): δ = 1.39 (s, 3 H, acetonide-Me), 1.42 (s, 3 H, acetonide-Me), 3.02 (d, *J* = 11.9 Hz, 1 H, 5-OH), 3.84 (s, 3 H, COOMe), 3.96 (dddd, *J* = 11.9, 4.9, 3.1, 1.1 Hz, 1 H, H5), 4.56 (ddd, *J* = 5.9, 3.1, 1.1 Hz, 1 H, H4), 4.77 (ddd, *J* = 5.9, 3.5, 0.9 Hz, 1 H, H3), 5.10 (t, *J* = 4.9, 0.9 Hz, 1 H, H6), 6.92 (dd, *J* = 3.5, 0.9 Hz, 1 H, H2).

HRMS: *m/z* $[M + H]^+$ calcd for $C_{11}H_{16}{}^{35}ClO_5$: 263.0685; found: 263.0693.

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