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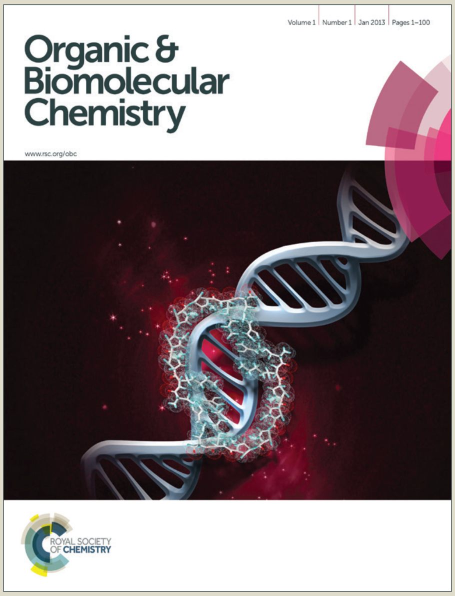
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ARTICLE

Enantioselective Total Syntheses of the Proposed Structures of Prevezol B and Evaluation of Anti-Cancer Activity

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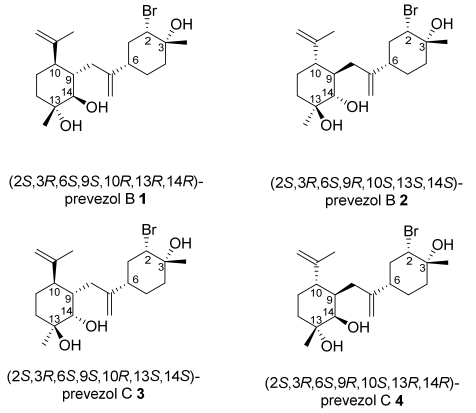
The first enantioselective total syntheses of the proposed structures of the natural product prevezol B are reported. The reported syntheses complement the previously-reported syntheses of the proposed structures of prevezol C, a stereoisomer of prevezol B. It was previously shown that the structure of the naturally occurring prevezol C had been incorrectly assigned. This work has led us to conclude that the proposed structures of prevezol B are also incorrect and major revision of both of the structures of the prevezols B and C is required. Cytotoxicity studies on the human cervical cancer cell line HeLa revealed that the synthesized prevezol B and C compounds were not active even at the highest concentration used (100 μM). However, one of the synthetic precursors was shown to have modest potency against the HeLa cells ($IC_{50} = 23.5 \pm 1.8$ μM).

Introduction

The isolation of the natural product family known as the prevezols was reported over a decade ago by Roussis and co-workers.[1, 2] The structures and relative stereochemistry of the prevezol family of natural products were determined by analysis of the spectroscopic data. Their molecular frameworks were found to be unlike any that had been reported previously. Our group has been particularly interested in the prevezols B and C, which were reported to be diastereomers of one another, because they exhibit activity (in the micromolar range) against a range of human tumour cell lines.[2] The absolute configuration of the prevezols B and C remained undefined when their isolation was reported, and thus two diastereomers (**1** and **2** for prevezol B, and **3** and **4** for prevezol C, Figure 1) and their enantiomers were proposed for each of the natural products.



(2*S*,3*R*,6*S*,9*S*,10*R*,13*R*,14*R*)-prevezol B **1**

(2*S*,3*R*,6*S*,9*R*,10*S*,13*S*,14*S*)-prevezol B **2**

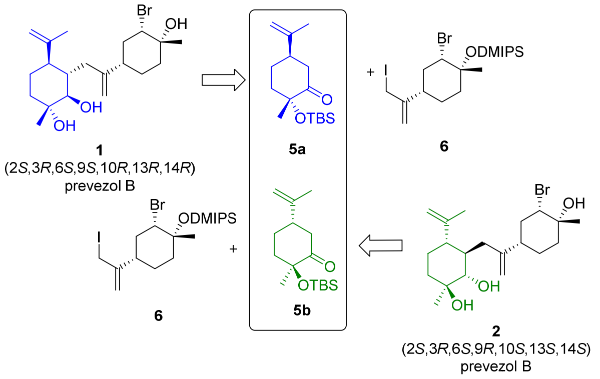
(2*S*,3*R*,6*S*,9*S*,10*R*,13*S*,14*S*)-prevezol C **3**

(2*S*,3*R*,6*S*,9*R*,10*S*,13*R*,14*R*)-prevezol C **4**

Figure 1: The reported structures of two of the proposed diastereomers of prevezol B (**1** and **2**) and prevezol C (**3** and **4**) (numbered in accordance with Roussis and co-workers).[2]

Over the past several years we have developed methodology for construction of the novel carbon skeleton common to the prevezols B and C, and recently we published the first asymmetric synthesis of two of the proposed structures of prevezol C, **3** and **4**.[3] It was discovered, via analysis of the ^1H and ^{13}C NMR spectroscopic data, that neither diastereomer (nor the corresponding enantiomer) of the proposed structure was consistent with the data obtained for the natural product.[3] Since the prevezols B and C were deemed to be stereoisomers of one another, differing only in the stereochemistry of the 1,2-diol,[2] we anticipated that the synthesis of the proposed structures of prevezol B could be conducted in a similar fashion to the diastereomers of prevezol C. It was hoped that synthesis of these diastereomers would allow absolute structural elucidation of prevezol B and enable the structure of prevezol C to be corrected.

The retrosynthetic strategy for the targets **1** and **2** is shown in Scheme 1. We anticipated that each of the targets could be accessed via diastereoselective alkylation reactions between the required enantiomer of the TBS ketone (**5a** or **5b**), and the allylic iodide **6**[3].



Scheme 1: Retrosynthetic analysis of the reported structures of the proposed diastereomers of prevezol B (**1** and **2**), Structures are numbered in accordance with Roussis and co-workers.[2]

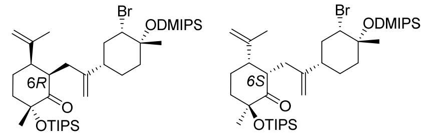
Herein, we report the first total asymmetric syntheses of the proposed diastereomers of prevezol B **1** and **2**, employing synthetic methodology established within our group.[3] We also disclose the cytotoxicity of the synthesized structures of prevezol B, prevezol C and a number of synthetic precursors against the human cervical cancer cell line HeLa.

Results and Discussion

Synthesis

The required TBS ketone **5a** (structure in Scheme 1) was synthesized with high enantiopurity in three steps from (+)-limonene oxide, via the hydroxyketone **7a**, using a combination of published procedures describing the preparation of its enantiomer.[4, 5] Unfortunately, reaction of the protected ketone **5a** with the allylic iodide **6**[3] resulted in low yields of the coupled product; the major product was the deprotected hydroxyketone **7a**.[†] The use of the more stable TIPS protecting group was thus investigated (Scheme 2).

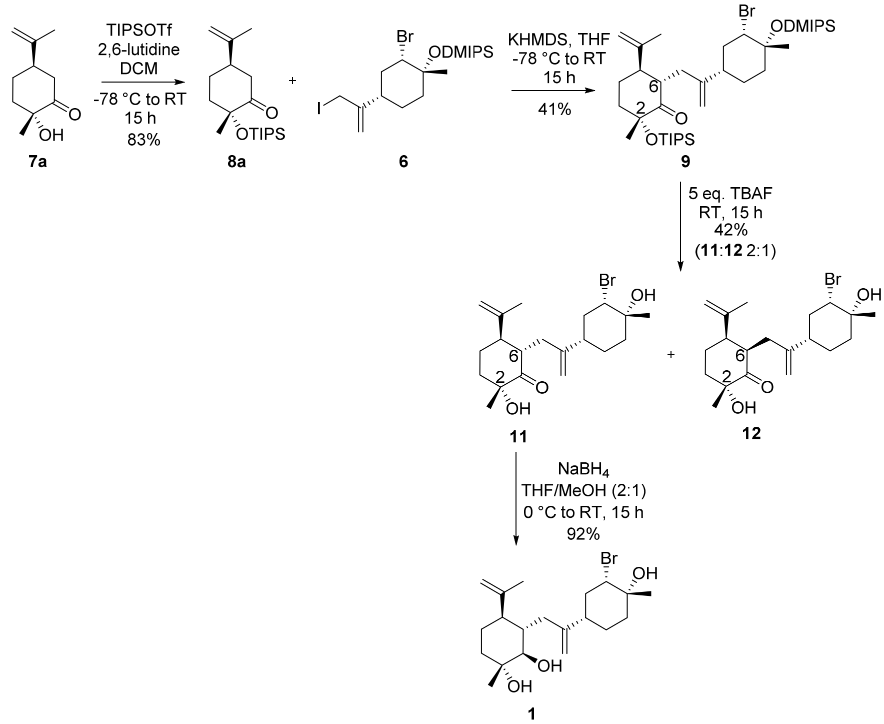
Reaction of the hydroxyketone **7a** with triisopropylsilyl trifluoromethanesulfonate (TIPSOTf)[6] and 2,6-lutidine in dry DCM gave the TIPS-protected compound **8a** in good yield. Reaction of the ketone **8a** with the allylic iodide **6** provided the diterpene **9** with good diastereomeric control; occasionally up to 10% of the undesired 6*R*[∫] epimer **10** (Figure 2) was observed. Under these reaction conditions cleavage of the TIPS ether was not observed.



10 **14**

Figure 2: Undesired epimers formed during alkylation reactions.

The alkylated centre was deduced to be *S* via coupling constant analysis (H6, ddd, J = 12.0, 10.0, 2.0 Hz). A global deprotection (5 equiv. TBAF) of diastereomerically pure **9** produced a 2:1 ratio of the desired product **11** and its epimer **12** (Scheme 2). Identification of each diastereomer was achieved by coupling constant analysis of the proton at C6 in addition to an evaluation of the chemical shift of these protons: axial protons in cyclohexane systems are reported to occur at higher field than chemically similar, but equatorially-positioned protons.[7] The resonance at 2.92 ppm (coalesced ddd, J = 12.2, 10.3, 1.9 Hz) was attributed to the proton at the alkylated centre of compound **11** (assigned as *S* at this centre) and the resonance at 3.19 ppm (apparent dt, J = 9.6, 5.6 Hz) was attributed to the alkylated centre of the epimeric, undesired diterpene diol **12**, assigned as *R* at the alkylated centre (Figure 3). The epimerisation of C6 was unexpected as this phenomenon had not been observed during the synthesis of the reported structures of prevezol C **3** and **4**,[3] and thus it appears that the configuration of the C2 centre dramatically affects the stability of the diterpene diol **11**. Though it is known that the use of TBAF can cause inversion of an asymmetric centre adjacent to a ketone,[8] surprisingly, the observed transformation seemed to allow formation of the less stable isomer **12** (with the large alkyl group in the axial position). The desired diastereomer **11** could be isolated by careful silica gel chromatography from the initial mixture, albeit in low yields (12%). Reaction of the diastereomerically pure diterpene diol **11** with sodium borohydride in a THF/methanol mixture cleanly provided the 2*S*,3*R*,6*S*,9*S*,10*R*,13*R*,14*R* diastereomer, compound **1**. This compound was found to be highly unstable to silica gel chromatography, which was distinct from both of its previously synthesized diastereomers (compounds (2*S*,3*R*,6*S*,9*S*,10*R*,13*S*,14*S*)-**3** and (2*S*,3*R*,6*S*,9*S*,10*S*,13*R*,14*R*)-**4**).[3] Fortunately, the crude product was of sufficient purity for analysis, including comprehensive 2D NMR spectroscopic analysis (see SI), which allowed the ^{1}H and ^{13}C NMR spectra to be unambiguously assigned. The spectroscopic data for compound **1** did not match the data obtained for the natural product.



Scheme 2: Synthesis of the reported structure of prevezol B **1**.

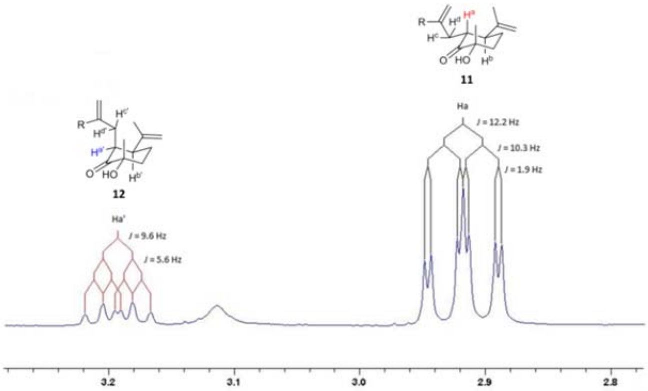
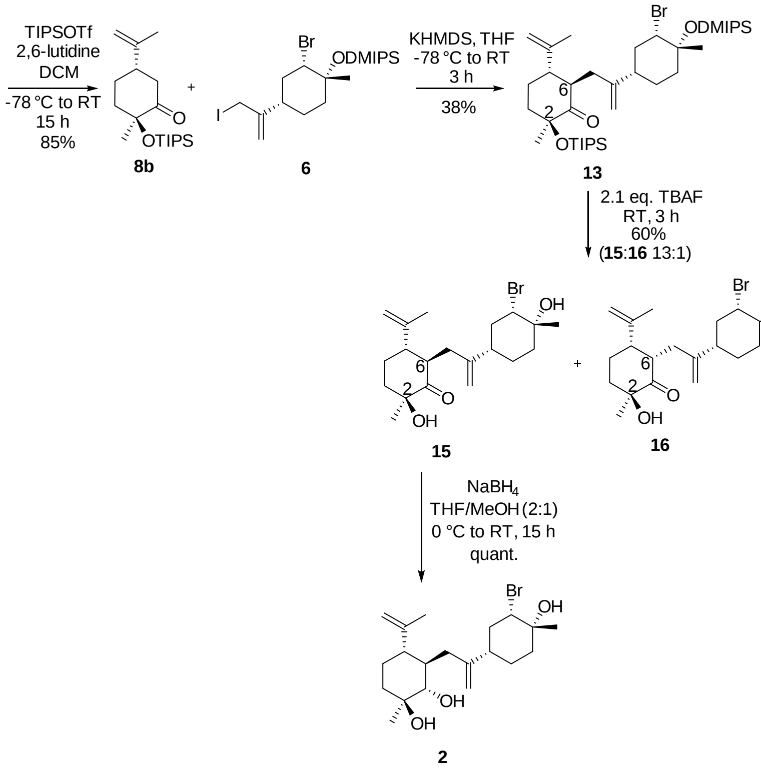


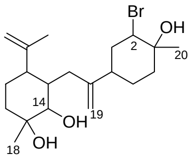
Figure 3: The ^{1}H NMR spectroscopic resonances (600 MHz, $CDCl_3$) attributed to the methine protons (H6) of the alkylated centres of compounds **11** and **12**.

The alternative reported structure of prevezol B, **2**, was synthesized in a similar manner. Due to the labile nature of the TBS protecting group of compound **5b**, the corresponding TIPS ketone **8b** was synthesized in good yield from the hydroxyketone **7b** using TIPSOTf and 2,6-lutidine (Scheme 3). Alkylation of the TIPS ketone **8b** with the allylic iodide **6** gave the diterpene with good diastereomeric control; occasionally up to 10% of the undesired *S* epimer **14** (Figure 2) was observed. Though an excess of the allylic iodide **6** was used, complete consumption of the TIPS ketone **8b** was not observed. Treatment of diastereomerically pure **13** (contaminated with 16% of the TIPS ketone **8b**) with 2.1 equivalents of TBAF gave a 13:1 ratio of the desired product **15** and its epimer **16**, in 60% yield. No reaction was observed when attempts were made to deprotect **13** using HF. Fortunately, the diastereomerically pure diol could be obtained after careful silica gel chromatographic purification. Reduction with sodium borohydride in a THF/methanol mixture provided the (2*S*,3*R*,6*S*,9*R*,10*S*,13*S*,14*S*)-diastereomer **2** exclusively. Surprisingly, compound **2** proved to be highly unstable in $CDCl_3$. Storing the solvent over K_2CO_3 and analysing the sample without delay allowed collection of ^{1}H NMR spectroscopic data, but was unsuitable for experiments requiring longer acquisition times. Thus, NMR spectroscopic data for this compound was typically obtained in C_6D_6. . Comparison of the ^{1}H NMR spectrum obtained in $CDCl_3$ with that of the natural product revealed that it did not match the spectroscopic profile of prevezol B reported by Roussis and co-workers.[2,‡]

As a result of this work, we do not believe that the structural assignment of prevezol B is correct. To highlight this, a number of key ^{1}H NMR spectroscopic signals for the synthesized compounds **1** and **2** and the previously synthesized diastereomers[3] (compounds **3** and **4**), as well as those observed for the natural products are summarized in Table 1. It can be seen that whilst the chemical shifts of the C20 methyl group and the methine proton at C2 in the synthesized analogs are consistent with the corresponding chemical shifts observed in the natural products, the chemical shifts of the C18 methyl group and the C14 methine proton resonances differ significantly.



Scheme 3: Synthesis of the reported structure of prevezol B **2**.



	δ_H					
	Naturally occurring prevezol B[2]	Naturally occurring prevezol C[2]	**1**	**2**	**3**[3]	**4**[3]
H2	4.13 (dd, 6.6, 10.0)	4.13 (dd, 4.1, 12.3)	4.14 (dd, 4.4, 12.0)	4.18 (m)	4.15 (dd, 4.4, 12.0)	4.19 (dd, 4.4, 12.3)
H14	4.02 (d, 11.2)	3.60 (d, 4.4)	3.34 (d, 10.1)	3.34 (d, 10.0)	3.36 (s)	3.36 (s)
H18	1.40 (s)	1.60 (s)	1.22 (s)	1.38 (s)	1.27 (s)	1.28 (s)
$H19_a$	4.87 (br s)	4.88 (br s)	4.89 (s)	4.95 (s)	4.89 (s)	4.89 (s)
$H19_b$	4.80 (br s)	4.86 (br s)	4.82 (s)	4.75 (s)	4.82 (s)	4.81 (s)
H20	1.31 (s)	1.31 (s)	1.34 (s)	1.23 (s)	1.33 (s)	1.33 (s)

[a] All spectra were recorded in $CDCl_3$. Chemical shifts are expressed in ppm. *J* values in parentheses are in Hz. Hydrogen atoms numbered in accordance with Roussis and co-workers.[2]

Table 1: Selected ^{1}H NMR spectroscopic data for naturally occurring prevezol B and C, and compounds **1-4**.[a]

Cytotoxicity studies

Whilst the spectroscopic data of the synthesized compounds did not match the spectroscopic data reported for the natural products, it was of interest to evaluate the cytotoxicity of a number of final products and synthetic precursors. The results are summarized in Table 2. Roussis and co-workers reported that naturally occurring prevezol B and C showed cytotoxicity values of 78.0 and 80.5 μM, respectively, against the human cervical cancer cell line HeLa.[2] None of the synthesized diterpene analogs[3], aside from compound **17**, showed any activity (>100 μM) in our assay. The most potent compound was found to be the DMIPS-protected compound **17**, which has an slightly higher IC_{50} value (IC_{50} = 23.5 ± 1.8 μM) than that of the well-established anticancer drug cisplatin **20** (11.5 ± 2.9 μM). Interestingly, neither the TBS-protected analog of **17** (compound **18**), nor the deprotected analog **19** had any activity.

Conclusion

In conclusion, total synthesis has allowed us to show that the spectroscopic data for the structures proposed by Roussis and co-workers for prevezol B[1, 2] do not match those reported for the natural product. Along with the results of our previous work,[3] this indicates that the prevezol family of compounds remains structurally ill-defined and that further work will be required to determine their precise identity. More generally, this study once again highlights the value of total synthesis as a means of proving/disproving structures proposed on the basis of spectroscopic studies of natural products.

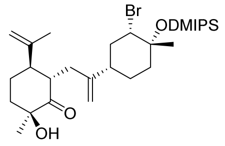
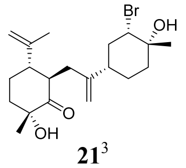
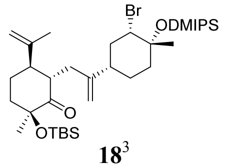
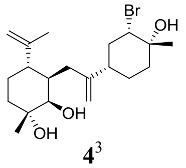
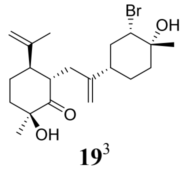
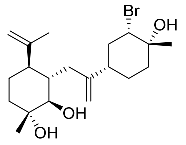
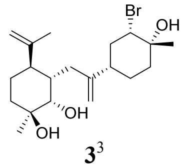
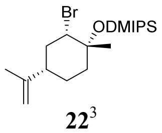
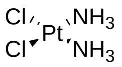
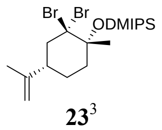
Compound	**IC_{50} values (μM)**	**Compound**	**IC_{50} values (μM)**
 **17**	23.5 ± 1.8	 **21**[3]	>100
 **18**[3]	>100	 **4**[3]	>100
 **19**[3]	>100	 **1**	>100
 **3**[3]	>100	 **22**[3]	70.0 ± 17.4
 **20**	11.5 ± 2.9	 **23**[3]	55.5 ± 2.3

Table 2: Cytotoxicity studies, with HeLa cells, of compounds **1**, **3, 4**, **17-19**, **21-23**; cisplatin **20** was used as positive control; the results are expressed as mean ± standard deviation of at least three independent experiments.

Experimental

General Experimental

Triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) was prepared according to literature procedures.[6, 9] All other reagents were used as received from the manufacturer. Solvents were dried, when necessary, by standard methods. Organic extracts were dried over $MgSO_4$. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on either a Bruker AV400 or Bruker AV600 spectrometer operating at 400 and 600 MHz respectively for proton and 100 and 150 MHz respectively for carbon nuclei. Chemical shifts (δ) are expressed in parts per million (ppm) and are referenced to residual solvent signal as the internal standard. Infrared spectra were recorded on an Agilent Cary 630 FTIR as a solution, or neat sample. Optical rotations were determined using a PolAAR 2001 automatic polarimeter, using a 1 dm cell with chloroform or ethanol as solvent, at a wavelength of 589 nm (sodium D line). Specific rotations are reported based on the equation $[\alpha] = (100\times\alpha)/(l\times c)$ where concentration c is in g/100 mL and path length l is in decimetres. High resolution mass spectra (HRMS) were recorded on an Agilent 6220 accurate mass LC-TOF using purine/HP0921 mix as the reference compound.

Synthesis

(2*R*,5*R*)-2-Methyl-5-(prop-1-en-2-yl)-2-((triisopropylsilyl)oxy)cyclohexanone (8a).

A solution of the hydroxyl ketone **7a**[5] (570 mg, 3.39 mmol) in dry DCM (60 mL) was cooled to −78 °C. 2,6-Lutidine (1.00 mL, 8.63 mmol) and TIPSOTf (2.30 mL, 8.56 mmol) were added dropwise, and the reaction mixture was allowed to warm to rt slowly over 15 h, then quenched with 1 M HCl (40 mL). The mixture was partitioned, and the organic layer was washed with brine (3 × 40 mL), dried and concentrated to provide the crude product as a pink oil. Flash column chromatography (2% EtOAc in hexane) provided the pure title compound as a clear oil (912 mg, 83%). $[\alpha]_D^{20} = +21.3$ (c 1.07 in $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.04 (complex, 18 H), 1.11 (m, 3 H), 1.40 (s, 3 H), 1.67 (m, 1 H), 1.73 (s, 3 H), 1.86 (m, 1 H), 1.91-2.03 (complex, 2 H), 2.41-2.49 (complex, 2 H), 2.61 (m, 1 H), 4.73 (s, 1 H), 4.80 (s, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.8, 18.66, 18.67, 21.2, 25.6, 27.2, 40.8, 43.4, 45.5, 78.9, 110.7, 147.0, 210.7. IR (neat): 2939 (s), 2863 (s), 1724 (s), 1645 (w), 1460 (m), 1373 (m), 1208 (s), 1142 (s), 1053 (s), 1014 (m), 881 (s), 773 (s), 671 (s) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{19}H_{37}O_2Si^+$ $[M+H]^+$ 325.2557, found 325.2557.

(2*R*,5*R*,6*S*)-6-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-((isopropyldimethylsilyl)oxy)-4-methylcyclohexyl)allyl)-2-methyl-5-(prop-1-en-2-yl)-2-((triisopropylsilyl)oxy)cyclohexanone (9).

To a −78 °C solution of TIPS ketone **8a** (75 mg, 0.23 mmol) in dry THF (8 mL) was added a solution of KHMDS (0.50 M in toluene, 1.1 mL, 0.55 mmol). The mixture was stirred at this temperature for 30 min, and then a solution of crude allylic iodide **6**[3] (0.12 g, 0.27 mmol) in dry THF (4 mL) was added. The mixture was allowed to warm to rt over 15 h, and was subsequently quenched with sat. aq. NH_4Cl (15 mL), washed with brine (2 × 25 mL), dried and concentrated to afford the crude compound as a yellow oil. Flash column chromatography (2% EtOAc in hexane) provided the pure title compound as a clear oil (62 mg, 41%), usually as a single diastereomer, but sometimes contaminated with a small amount (<10%) of its C6 epimer **10**. $[\alpha]_D^{20} = -7.0$ (c 1.0 in $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (s, 3 H), 0.15 (s, 3 H), 0.76 (m, 1 H), 0.98-1.02 (complex, 24 H), 1.07-1.13 (complex, 3 H), 1.36 (s, 3 H), 1.43 (apparent td, *J* = 13.4, 4.0 Hz, 1 H), 1.52 (s, 3 H), 1.56-2.21 (complex, 15 H), 2.39 (dd, *J* = 16.0, 10.0 Hz, 1 H), 2.76 (ddd, *J* = 12.0, 10.0, 2.0 Hz, 1 H), 3.90 (dd, *J* = 12.4, 4.0 Hz, 1 H), 4.44 (s, 1 H), 4.71 (s, 1 H), 4.75 (s, 1 H), 4.82 (t, *J* = 1.6 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ −1.1, −0.8, 14.0, 16.3, 17.3, 17.4, 18.4, 18.78, 18.83, 25.5, 26.9, 29.1, 30.1, 30.7, 39.0, 39.9, 42.8, 46.6, 49.2, 54.3, 64.8, 73.5, 80.0, 107.2, 113.2, 145.8, 152.3, 210.6. IR (neat): 3075 (w), 2936 (s), 2861 (s), 1723 (s), 1641 (m), 1460 (m), 1374 (m), 1325 (w), 1250 (m), 1199 (m), 1173 (m), 1138 (m), 1050 (s), 993 (m), 881 (s), 733 (s) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{34}H_{64}{}^{81}BrO_3Si_2{}^+$ $[M+H]^+$ 657.3551, found 657.3568; calcd for $C_{34}H_{64}{}^{79}BrO_3Si_2{}^+$ $[M+H]^+$ 655.3577, found 655.3575.

(2*R*,5*R*,6*S*)-6-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-hydroxy-4-methylcyclohexyl)allyl)-2-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone (11).

To a solution of the diastereomerically-pure diterpene **9** (40 mg, 61 μmol) in dry THF (9 mL) was added a solution of TBAF (1.0 M in THF, 300 μL, 300 μmol). The mixture was allowed to stir at rt for 15 h, and was then filtered through a silica plug to provide the crude title compound as the major constituent, along with its C6 epimer **12**, in a 2:1 ratio. Flash column chromatography (20% EtOAc in hexane) allowed partial separation of these diastereomers, to provide the pure title compound as a clear oil (3 mg, 12%) and a mixture of the title compound **12** and its C6 epimer **11** as a clear oil (7 mg, 1:1 ratio, 30%). Compound **11**: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 3 H), 1.45-1.53 (complex, 4 H), 1.58-2.20 (complex, 15 H), 2.36 (dd, *J* = 16.4, 10.4 Hz, 1 H), 2.92 (ddd, *J* = 12.3, 10.4, 1.9 Hz, 1 H), 4.17 (m, 1 H), 4.46 (s, 1 H), 4.76 (s, 1 H), 4.78 (s, 1 H), 4.86 (t, *J* = 1.5 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 18.4, 24.4, 26.8, 29.0, 30.6, 30.71, 37.81, 39.2, 41.9, 46.7, 47.7, 54.7, 66.0, 70.49, 76.6, 107.2, 113.6, 145.3, 151.2, 214.16. IR ($CHCl_3$ solution): 3480 (br, m), 3075 (w), 2967 (s), 2930 (s), 2860 (s), 1707 (s), 1643 (m), 1451 (s), 1375 (s), 1260 (m), 1187 (m), 1164 (m), 1101 (m), 1001 (m), 924 (m), 895 (s), 804 (m) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{20}H_{32}{}^{81}BrO_3{}^+$ $[M+H]^+$ 401.1509, found 401.1503; calcd for $C_{20}H_{32}{}^{79}BrO_3{}^+$ $[M+H]^+$ 399.1529, found 399.1520.

Data for **(2*R*,5*R*,6*R*)-6-(2-((1*S*,3*S*,4*R*)-3-bromo-4-hydroxy-4-methylcyclohexyl)allyl)-2-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone (12)**: ^1H NMR ($CDCl_3$, 600 MHz) δ 1.33 (s, 3 H), 1.36 (s, 3 H), 1.46-2.19 (complex, 15 H) 2.38 (dd, *J* = 15.3, 9.8 Hz, 1 H), 2.56 (apparent quint, *J* = 4.3 Hz, 1 H), 3.12 (br s, 1 H), 3.19 (apparent dt, *J* = 9.8, 5.5 Hz, 1 H), 4.08 (br s, 1 H), 4.17 (dd, *J* = 12.1, 4.6 Hz, 1 H), 4.64 (s, 1 H), 4.76 (s, 1 H), 4.83 (s, 1 H), 4.94 (s, 1 H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 23.1, 23.5, 25.7, 27.0, 30.69, 32.1, 37.77, 38.7, 39.2, 44.3, 48.0, 48.2, 65.8, 70.49, 75.7, 110.6, 112.9, 144.0, 149.5, 214.23. LC-MS (ESI^+) *m/z* calcd for $C_{20}H_{31}{}^{81}BrNaO_3{}^+$ $[M+Na]^+$ 423.1, found 423.1 (60%); calcd for $C_{20}H_{32}{}^{81}BrO_3{}^+$ $[M+H]^+$ 401.1, found 401.1 (40%).

(1*R*,2*R*,3*S*,4*R*)-3-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-hydroxy-4-methylcyclohexyl)allyl)-1-methyl-4-(prop-1-en-2-yl)cyclohexane-1,2-diol (1).

To a 0 °C solution of the diterpene diol **11** (3.8 mg, 10 μmol) in a mixture of THF/MeOH (2:1, 3 mL) was added $NaBH_4$ (5 mg, 0.13 mmol). The reaction mixture was warmed to rt over 15 h, then quenched with sat. aq. $NaHCO_3$ (15 mL) and extracted into ether (2 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (30 mL), then brine (30 mL), dried and concentrated to provide the title compound as a white solid, which typically required no further purification (and which was unstable on silica gel) (3.5

mg, 92%). $[\alpha]_D^{20} = -28.6$ (c 0.275 in $CHCl_3$). ^1H NMR ($CDCl_3$, 600 MHz) δ 1.22 (s, 3 H, H18), 1.34 (s, 3 H, H20), 1.43-1.77 (complex, 11 H, H4a, H5, H9, H11a, H12 and H17), 1.85-1.96 (complex, 2 H, H8a and H10), 2.03-2.21 (complex, 4 H, H1, H4b and H6), 2.37 (m, 1 H, H8b), 3.34 (d, *J* = 10.1, 1 H, H14), 4.14 (dd, *J* = 12.0, 4.4 Hz, 1 H, H2), 4.76 (m, 1 H, H16a), 4.82 (m, 1 H, H16b), 4.89 (s, 1 H, H19a), 4.95 (s, 1 H, H19b). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 19.3 (C17), 20.2 (C18), 26.3 (C5), 28.9 (C11), 30.7 (C20), 37.2 (C12), 37.7 (C4), 39.0 (C8), 39.7 (C1), 41.0 (C9), 44.9 (C6), 50.8 (C10), 65.7 (C2), 70.5 (C3), 73.4 (C13), 83.5 (C14), 110.0 (C19), 112.7 (C16), 147.2 (C15), 154.7 (C7). IR ($CHCl_3$ solution): 3451 (br, s), 3074 (w), 2968 (s), 2932 (s), 2863 (s), 1643 (m), 1451 (s), 1376 (s), 1260 (s), 1099 (s), 1076 (s), 1011 (s), 892 (s), 803 (s), 754 (s) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{20}H_{33}{}^{81}BrNaO_3{}^+$ $[M+Na]^+$ 425.1485, found 425.1482; calcd for $C_{20}H_{33}{}^{79}BrNaO_3{}^+$ $[M+Na]^+$ 423.1505, found 423.1501.

(2*S*,5*S*)-2-Methyl-5-(prop-1-en-2-yl)-2-((triisopropylsilyl)oxy)cyclohexanone (8b).

To a −78 °C solution of the hydroxyl ketone **7b**[5] (187 mg, 1.11 mmol) in dry DCM (20 mL) was added 2,6-lutidine (410 µL, 3.54 mmol) and TIPSOTf (630 µL, 2.34 mmol) slowly. The reaction mixture was allowed to warm to rt over 15 h, then quenched with 1 M HCl (20 mL) and partitioned. The organic extract was washed with brine (3 × 15 mL), dried and concentrated to afford the crude title compound as an orange oil. Flash column chromatography (2% EtOAc in hexane) provided the pure title compound as a clear oil (307 mg, 85%). $[\alpha]_D^{20} = -20.9$ (c 1.04 in $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.02-1.07 (complex, 18 H), 1.08-1.18 (complex, 3 H), 1.40 (s, 3 H), 1.65 (m, 1 H), 1.74 (s, 3 H), 1.83-2.03 (complex, 3 H), 2.41-2.50 (complex, 2 H), 2.57-2.65 (complex, 2 H), 4.73 (d, *J* = 0.4 Hz, 1 H), 4.80 (t, *J* = 1.2 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.8, 18.65, 18.67, 21.2, 25.6, 27.2, 40.8, 43.4, 45.5, 78.9, 110.7, 147.0, 210.7. IR (neat): 3086 (w), 2941 (s), 2864 (s), 1725 (s), 1646 (w), 1461 (s), 1376 (m), 1208 (s), 1173 (s), 1142 (s), 1098 (m), 1055 (s), 881 (s), 773 (s) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{19}H_{36}NaO_2Si^+$ $[M+Na]^+$ 347.2377, found 347.2381.

(2*S*,5*S*,6*R*)-6-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-((isopropyldimethylsilyl)oxy)-4-methylcyclohexyl)allyl)-2-methyl-5-(prop-1-en-2-yl)-2-((triisopropylsilyl)oxy)cyclohexanone (13).

To a −78 °C solution of the TIPS ketone **8b** (54 mg, 0.17 mmol) in dry THF (8 mL) was added a solution of KHMDS (0.50 M in toluene, 0.85 mL, 0.43 mmol). The reaction mixture was kept at this temperature for 30 min and then a solution of the crude allylic iodide **6**[3] (0.11 g, 0.23 mmol) in dry THF (4 mL) was added dropwise. The mixture was allowed to warm to rt over 3 h, then quenched with sat. aq. NH_4Cl (15 mL) and extracted into ether (2 × 15 mL). The organic extracts were washed with brine (3 × 20 mL), dried and concentrated to afford the crude title compound as a yellow oil. Flash column chromatography (2% EtOAc in hexane) provided the pure title compound as a clear oil (42 mg, 38%), usually as a single diastereomer, but sometimes contaminated with a small amount of its C6 epimer **14**. $[\alpha]_D^{20} = -23.8$ (c 1.12 in $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (s, 3 H), 0.15 (s, 3 H), 0.76 (sept, *J* = 7.2 Hz, 1 H), 0.98-1.02 (complex, 24 H), 1.06-1.17 (complex, 3 H), 1.36 (s, 3 H), 1.43 (apparent td, *J* = 13.6, 3.6 Hz, 1 H), 1.49-1.52 (complex, 4 H), 1.62-2.14 (complex, 14 H), 2.36 (dd, *J* = 16.0, 10.0 Hz, 1 H), 2.78 (m, 1 H), 3.91 (m, 1 H), 4.44 (s, 1 H), 4.70 (s, 1 H), 4.75 (s, 1 H), 4.82 (t, *J* = 1.6 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ −1.1, −0.8, 14.0, 16.3, 17.3, 17.4, 18.4, 18.77, 18.83, 25.5, 26.6, 29.2, 30.1, 31.0, 39.4, 39.8, 42.8, 46.6, 49.1, 54.2, 64.8, 73.5, 80.0, 106.9, 113.1, 145.8, 152.2, 210.5. IR (neat): 3077 (w), 2926 (s), 2863 (s), 1726 (m), 1645 (w), 1461 (m), 1375 (m), 1251 (m), 1201 (m), 1177 (m), 1138 (m), 1051 (s), 881 (s), 802 (s), 772 (s) cm^{-1}. MS (ESI^+) *m/z* calcd for $C_{34}H_{63}{}^{81}BrNaO_3Si_2{}^+$ $[M+Na]^+$ 679.3, found 679.6 (100%); calcd for $C_{34}H_{63}{}^{79}BrNaO_3Si_2{}^+$ $[M+Na]^+$ 677.3, found 677.6 (95%); calcd for $C_{34}H_{62}NaO_3Si_2{}^+$ $[M-HBr+Na]^+$ 597.4, found 597.6 (50%). HRMS (ESI^-) *m/z* calcd for $C_{34}H_{62}{}^{81}BrNaO_3Si_2{}^-$ $[M-H]^-$ 655.3406, found 655.3418; calcd for $C_{34}H_{62}{}^{79}BrNaO_3Si_2{}^-$ $[M-H]^-$ 653.3426, found 653.3431.

(2*S*,5*S*,6*R*)-6-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-hydroxy-4-methylcyclohexyl)allyl)-2-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone (15).

To the diterpene **13**, containing 16% of the TIPS ketone **8b**, (23 mg, 29 µmol diterpene **13**) in THF (3 mL) was added a solution of TBAF (1.0 M in THF, 65 µL, 0.065 mmol). The mixture was stirred at rt for 3 h, then filtered through a silica plug, dried and concentrated. Compound **15**/**16** were present in a 1:13 ratio. Flash column chromatography (20% EtOAc in hexane) provided the pure title compound **15** (7 mg, 60%) as a clear oil. The epimeric diterpene diol **16** was isolated only as a 1:0.3 mixture with the title compound. Data for the title compound **15**: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 3 H), 1.45-1.53 (complex, 4 H), 1.58 (m, 1 H), 1.63-1.77 (complex, 6 H), 1.80-2.03 (complex, 3 H), 2.06-2.20 (complex, 5 H), 2.35 (dd, *J* = 16.4, 10.4 Hz, 1 H), 2.92 (ddd, *J* = 12.3, 10.3, 1.9 Hz, 1 H), 4.08 (br s, 1 H), 4.17 (dd, *J* = 11.6, 5.2 Hz, 1 H), 4.46 (s, 1 H), 4.76 (s, 1 H), 4.78 (s, 1 H), 4.86 (t, *J* = 1.6 Hz, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.4, 24.3, 26.5, 29.0, 30.6, 30.7, 37.7, 39.5, 42.0, 46.8, 47.8, 54.7, 66.0, 70.5, 76.6, 107.3), 113.6, 145.3, 151.2, 214.2. IR ($CDCl_3$ solution): 3475 (br, m), 3079 (w), 2931 (s), 2861 (m), 1708 (s), 1643 (m), 1450 (m), 1376 (s), 1319 (w), 1260 (w), 1228 (m), 1165 (m), 1101 (m), 1000 (m), 924 (m), 895 (s), 805 (m), 733 (m), 694 (m) cm^{-1}. HRMS (ESI^+) *m/z* calcd for $C_{20}H_{32}{}^{81}BrO_3{}^+$ $[M+H]^+$ 401.1509, found 401.1509; calcd for $C_{20}H_{32}{}^{79}BrO_3{}^+$ $[M+H]^+$ 399.1529, found 399.1528.

Data for **(2*S*,5*S*,6*S*)-6-(2-((1*S*,3*S*,4*R*)-3-bromo-4-hydroxy-4-methylcyclohexyl)allyl)-2-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohexanone (16)**: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s 3 H), 1.36 (s, 3 H), 1.48-2.16 (complex, 15 H), 2.40 (dd, *J* = 15.4, 9.2 Hz, 1 H), 2.57 (m, 1 H), 3.05 (br s, 1 H), 3.19 (apparent dt, *J* = 9.5, 5.7 Hz, 1 H), 4.16 (m, 1 H), 4.64 (m, 1 H), 4.76 (s, 1 H), 4.84 (s, 1 H), 4.95 (s, 1 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.2, 23.6, 25.7, 26.7, 30.7, 32.0, 37.8, 38.7, 39.7, 44.5, 48.0, 48.2, 65.8, 70.5, 75.7, 110.7, 112.9, 144.0, 149.6, 214.0. LC-MS (ESI^+) *m/z* calcd for $C_{20}H_{31}{}^{81}BrNaO_3{}^+$ $[M+Na]^+$ 423.1, found 423.1 (100%); calcd for $C_{20}H_{32}{}^{81}BrO_3$ $[M+H]^+$ 401.1, found 401.1 (40%).

(1*S*,2*S*,3*R*,4*S*)-3-(2-((1*S*,3*S*,4*R*)-3-Bromo-4-hydroxy-4-methylcyclohexyl)allyl)-1-methyl-4-(prop-1-en-2-yl)cyclohexane-1,2-diol (2).

To a 0 °C solution of the diterpene diol **15** (3.0 mg, 8 µmol) in a mixture of THF/MeOH (2:1, 3 mL) was added $NaBH_4$ (5 mg, 132 µmol). The reaction mixture was allowed to warm to rt over 15 h, then was quenched with sat. aq. $NaHCO_3$ and extracted into ether (2 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (20 mL), then brine (20 mL), dried and concentrated to provide the title compound as a white solid, which typically required no further purification (3 mg, quant.). $[\alpha]_D^{20} = -16.7$ (c 0.292 in $CDCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.23 (s, 3 H), 1.38 (s, 3 H), 1.42-2.41 (complex, 18 H), 3.34 (d, *J* = 10.0 Hz, 1 H), 4.18 (m, 1 H), 4.75 (m, 1 H), 4.80 (t, *J* = 1.7 Hz, 1 H), 4.88 (d, *J* = 1.0 Hz, 1 H), 4.95 (s, 1 H). ^1H NMR (C_6D_6, 600 MHz) δ 1.04 (m, 1 H, H4a), 1.09 (s, 3 H, H20), 1.19 (s, 3 H, H18), 1.24-1.39 (complex, 2 H, H11), 1.44-1.49 (complex, 2 H, H5a and H12b), 1.52 (s, 3 H, H17), 1.54-

1.76 (complex, 4 H, H5b, H9, H10 and H12a), 1.80 (dd, $J = 14.9, 7.9$ Hz, 1 H, H8a), 1.85 (dt, $J = 13.9, 3.2$ Hz, 1 H, H4b), 1.91 (tt, $J = 12.3, 3.0$ Hz, 1 H, H6), 1.99 (br s, OH), 2.16-2.40 (complex, 3 H, H1 and H8b), 3.18 (dd, $J = 9.9, 2.7$ Hz, 1 H, H14), 3.71 (dd, $J = 12.3, 4.4$ Hz, 1 H, H2), 4.70 (m, 1 H, H16a), 4.73 (s, 1 H, H19a), 4.75 (t, $J = 1.7$ Hz, 1 H, H16b), 4.81 (s, 1 H, H19b). ^{13}C NMR (C_6D_6, 150 MHz) δ 19.1 (C17), 20.2 (C18), 27.4 (C5), 29.2 (C11), 30.6 (C20), 37.6 (C12), 37.9 (C4), 38.9 (C8), 39.4 (C1), 41.1 (C9), 44.9 (C6), 51.1 (C10), 66.1 (C2), 70.1 (C3), 73.0 (C13), 83.1 (14), 109.6 (C19), 112.6 (C16), 147.5 (C15), 154.6 (C7). IR (C_6D_6 solution): 3454 (br, s), 3075 (w), 2970 (S), 2935 (s), 2865 (m), 1645 (m), 1456 (s), 1376 (m), 1135 (m), 1102 (m), 893 (m) cm^{-1}. HRMS (ESI$^+$) *m/z* calcd for $C_{20}H_{33}{}^{81}BrNaO_3^+$ $[M+Na]^+$ 425.1485, found 425.1511; calcd for $C_{20}H_{33}{}^{79}BrNaO_3^+$ $[M+Na]^+$ 423.1511, found 423.1506.

Cell Culture

Human cervical carcinoma cells (HeLa) cells were maintained in DMEM (Gibco) with 5% fetal calf serum (FCS, Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin at 37°C and 6% CO_2.

Cytotoxicity Studies

Cytotoxicity studies on human cervix HeLa cancer cell line treated with compounds **1**, **3**, **4** **17-19**, **21-23** were performed by a fluorometric cell viability assay using Resazurin (Promocell GmbH).[10] Briefly, one day before treatment cells were plated in triplicates in 96-well plates at a density of 4×10^3 cells / well in 100 μL. Stock solutions of the compounds were prepared in DMSO. Upon treating cells with increasing concentrations of the target compounds, the cells were incubated for 48 h at 37°C / 6% CO_2, the medium was then removed, and 100 μL of complete medium containing resazurin (0.2 mg / mL final concentration) was added. After 4 h of incubation at 37 °C / 6% CO_2, the fluorescence of the highly red fluorescent resorufin product was quantified at 590 nm emission with 540 nm excitation wavelength in a SpectraMax M5 microplate Reader. A series of negative controls with the cells untreated or treated just with the vehicle (DMSO, with a concentration < 0.5%) for each experiment were also performed. Cisplatin was used as positive control. The results are expressed as mean ± standard deviation of at least three independent experiments.

Human cervical carcinoma cells (HeLa) cells were maintained in DMEM (Gibco) with 5% fetal calf serum (FCS, Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin at 37°C and 6% CO_2.

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† The desired product was obtained, however the C6 epimer was also observed. Cleavage of the protecting group had not been observed in our previous work where we employed similar reaction conditions,[3] and thus it appears that the change in configuration at the C2 centre from axially- to equatorially-oriented dramatically affects the stability of the TBS ether.

∫ Prevezol B (**1** and **2**) and prevezol C (**3** and **4**) are numbered in accordance with Roussis and co-workers, however all other compounds are numbered according to IUPAC nomenclature.

‡ Work towards proposing alternative structures for the prevezols B and C was hindered by a lack of the natural samples, which have proven to be unstable to storage, and a lack of raw NMR spectroscopic data. We believe that more material needs to be isolated in order to allow for the proposal of new structures for these valuable natural products.

Electronic Supplementary Information (ESI) available: Experimental procedures for compound **17**, spectroscopic data for all new compounds; comparison of the ^{13}C NMR spectrum of **1** to data for the natural product. See DOI: 10.1039/b000000x/

(1) N. Mihopoulos, C. Vagias, E. Mikros, M. Scoullos and V. Roussis, *Tetrahedron Lett.*, 2001, **42**, 3749-3752.

(2) D. Iliopoulou, N. Mihopoulos, C. Vagias, P. Papazafiri and V. Roussis, *J. Org. Chem.*, 2003, **68**, 7667-7674.

(3) A. E. Leung, M. Blair, C. M. Forsyth and K. L. Tuck, *Org. Lett.*, 2013, **15**, 2198-2201.

(4) M. Blair, P. C. Andrews, B. H. Fraser, C. M. Forsyth, P. C. Junk, M. Massi and K. L. Tuck, *Synthesis*, 2007, **10**, 1523-1527.

(5) M. Blair and K. L. Tuck, *Tetrahedron: Asymmetry*, 2009, **20**, 2149-2153.

(6) E. J. Corey, H. Cho, C. Rücker and D. H. Hua, *Tetrahedron Lett.*, 1981, **22**, 3455-3458.

(7) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Am. Chem. Soc.*, 1958, **80**, 6098-6105.

(8) A. Nakamura, Y. Kaji, K. Saida, M. Ito, Y. Nagatoshi, N. Hara and Y. Fujimoto, *Tetrahedron Lett.*, 2005, **46**, 6373-6376.

(9) G. A. Olah, K. Laali and O. Farooq, *Organometallics*, 1984, **3**, 1337-1340.

(10) A. Leonidova, V. Pierroz, R. Rubbiani, Y. Lan, A. G. Schmitz, A. Kaech, R. K. O. Sigel, S. Ferrari and G. Gasser, *Chem. Sci.*, 2014, DOI: 10.1039/C3SC53550A.