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Highly antiproliferative, low-calcemic, side-chain ketone analogs of the hormone 1α,25-dihydroxyvitamin D_3

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Abstract—A series **2a**–**4b** of seven new side-chain ketone analogs of calcitriol (**1**) have been prepared. Unexpectedly, several of these 24- and 25-*tert*-butyl ketones, even though lacking the classical side-chain tertiary hydroxyl group, are considerably more antiproliferative in vitro than the hormone calcitriol (**1**) even at physiologically relevant low nanomolar concentrations and are less calcemic than calcitriol (**1**) in vivo. In addition, ketone analog 19-nor-**2a** is not significantly less calcemic in vivo than 19-methylene analog **2a**.

1. Introduction

For chemotherapy of various human illnesses, medicinal organic chemists are designing and preparing analogs that are at least as potent as, but less calcemic than, the natural hormone 1α,25-dihydroxyvitamin D_3 (**1**, calcitriol).[1–4] Currently, approximately eight such analogs of calcitriol (**1**) are regularly used as drugs that promote healthier living.[4] A continuing chemical challenge, however, is to determine which small structural changes and which simple functional group alterations in the large natural hormone molecule will elicit desirable biological responses. Understanding such chemical structure–biological activity relationships (SAR) is important for rational design of new analogs as potential drug candidates.[3,4] Toward this goal, we have developed a series of calcitriol analogs that lack the natural 25-OH group widely thought to be necessary for high biological activity.[1–3] Specifically, we rationally designed and prepared some side-chain sulfone[5] and sulfoximine[6] analogs such that these functional groups might act as H-bond acceptors (rather than as H-bond donors like the natural 25-OH group) toward the vitamin D receptor (VDR); several of these new analogs are potent, selective, and low-calcemic inhibitors of the human CYP24 hydroxylase enzyme.[5,6] Hoffman-La Roche researchers showed that incorporating a 16,17-double bond into an analog generally promotes antiproliferative activity.[7] Reddy and colleagues[8] showed that such a 16-ene 24-ketone 25-OH metabolite is even more antiproliferatively potent than its 24-CH_2 non-ketone parent, and we have similarly observed the high antiproliferative activity of a 16-ene 25-ketone analog.[9] However, as far as we know, there are no reported examples of any 16,17-saturated side-chain *tert*-butyl ketone analogs of calcitriol (**1**). Finally, DeLuca and colleagues[10] have reported recently a biologically active abbreviated side-chain analog having no side-chain heteroatom.

In this study, we describe a series of seven new 16,17-saturated analogs (**2a**–**4b**) of calcitriol (**1**) in which two minimal changes have been made in the side chain: (1) the natural side-chain tertiary hydroxyl group is replaced by a methyl group (forming a terminal *tert*-butyl group) and (2) a ketone carbonyl group is placed at either C-24 or C-25.

2. Results

2.1. Chemistry

Synthesis of new analogs **2a**, 19-nor-**2a**, and **3a** is outlined in Scheme 1, starting from previously reported side-chain iodides **5**[11] and **6**.[12] As noted before in the

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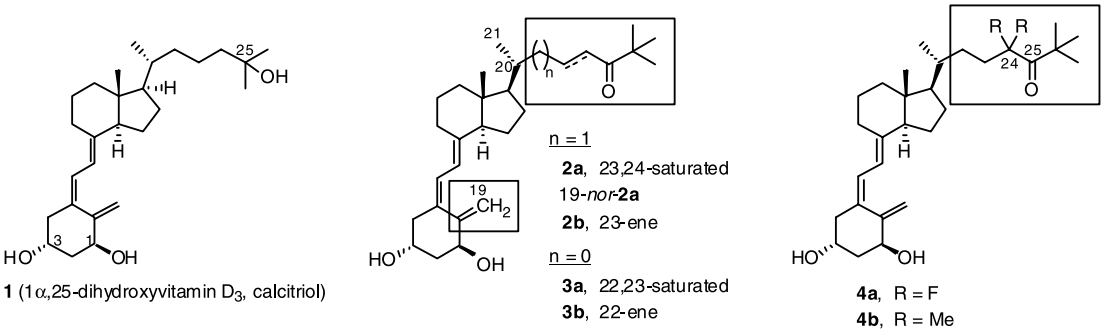
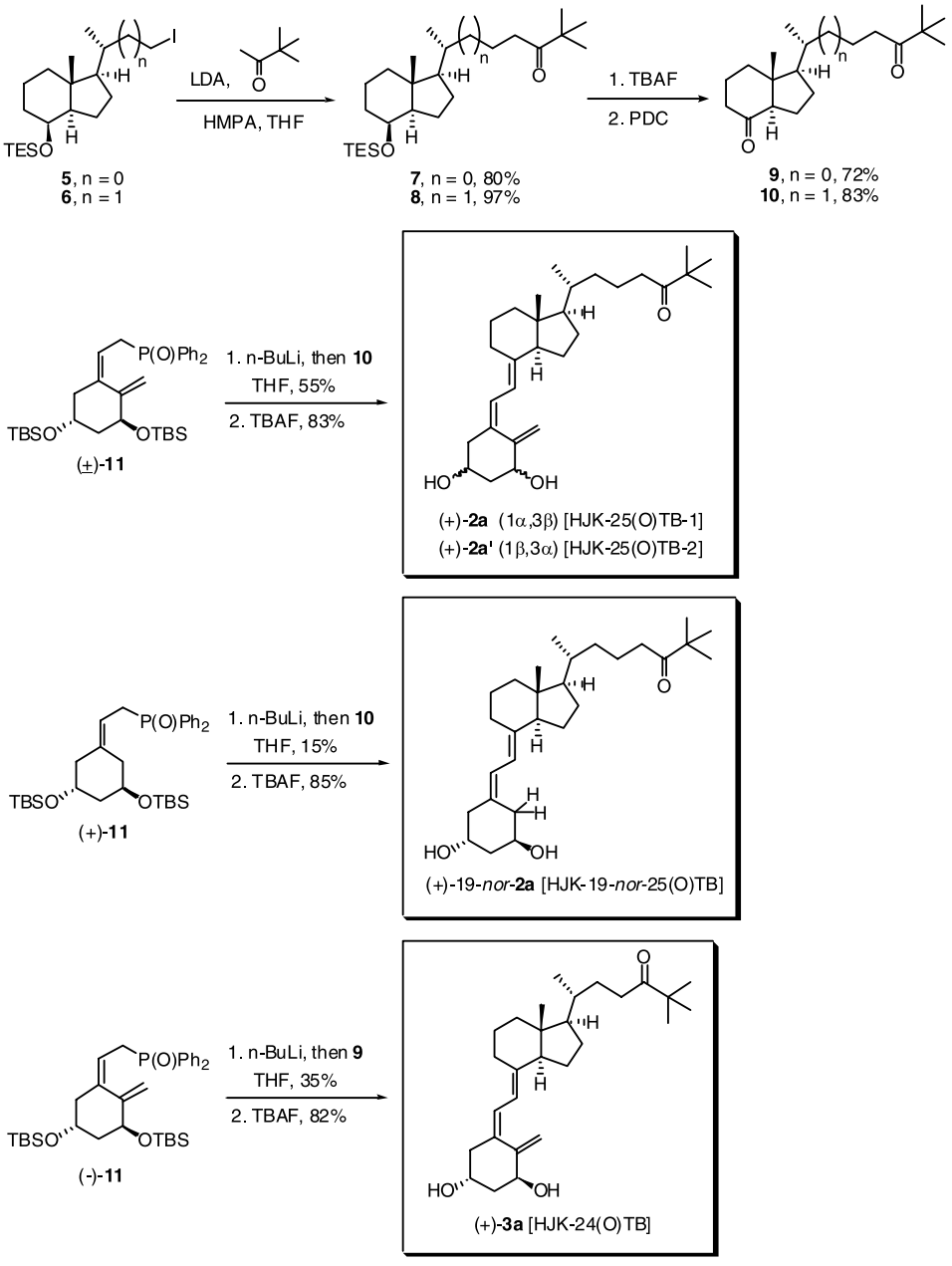
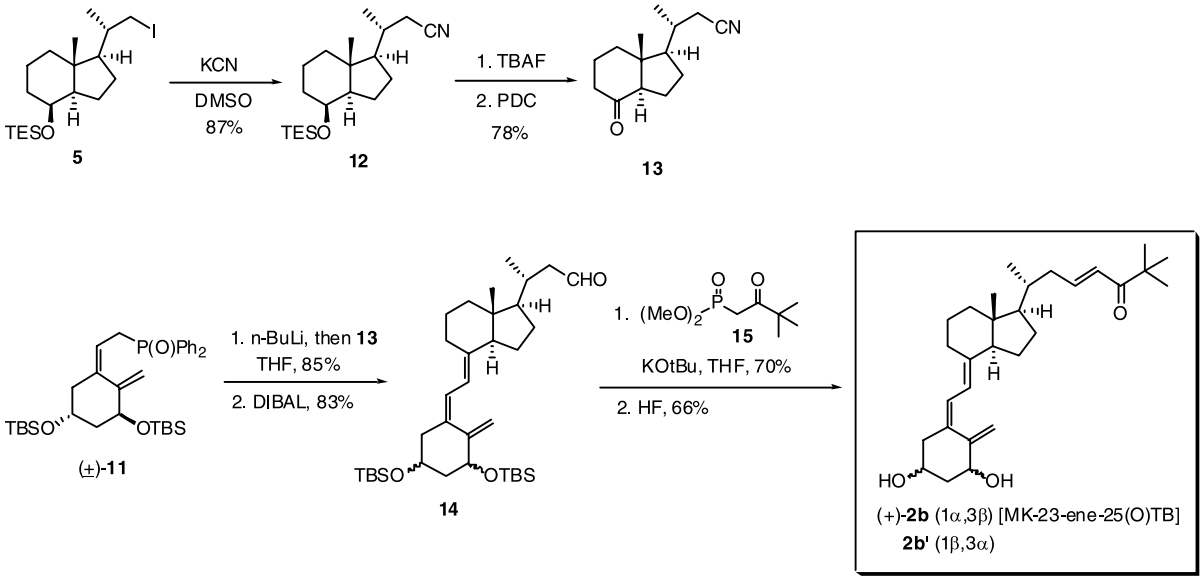


16-ene series,[12] so now in the 16,17-saturated series, Horner–Wadsworth–Emmons (HWE) coupling of the A-ring nucleophile with 8,24- and 8,25-diketones **9** and **10** proceeds regiospecifically at only C-8, the less-hindered ketone carbonyl group. Synthesis of new enone analog **2b** is summarized in Scheme 2, featuring



Scheme 1.



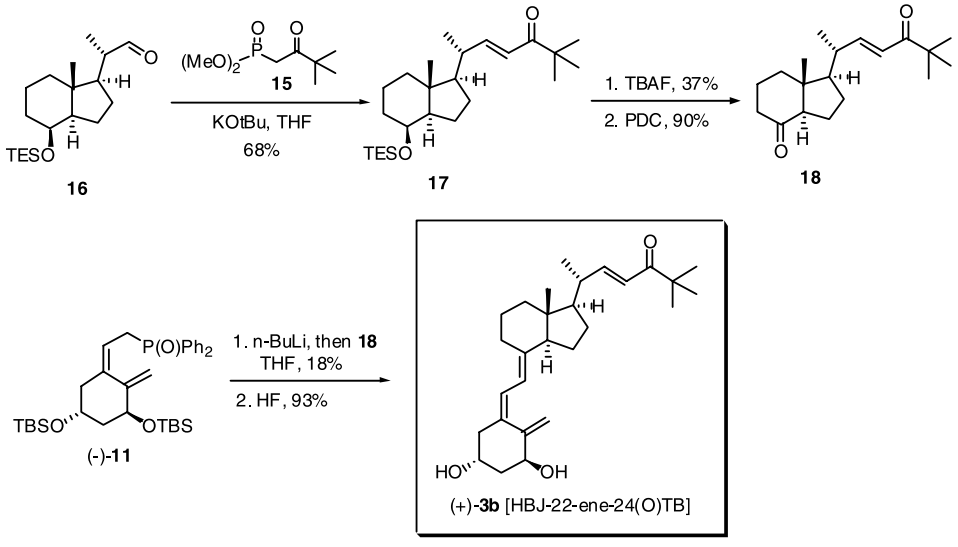
Scheme 2.

successful HWE coupling of triene aldehyde **14** without compromising the essential but sensitive conjugated triene unit. Synthesis of new analog **3b** is outlined in Scheme 3, and synthesis of analogs **4a** and **4b** is shown in Scheme 4.

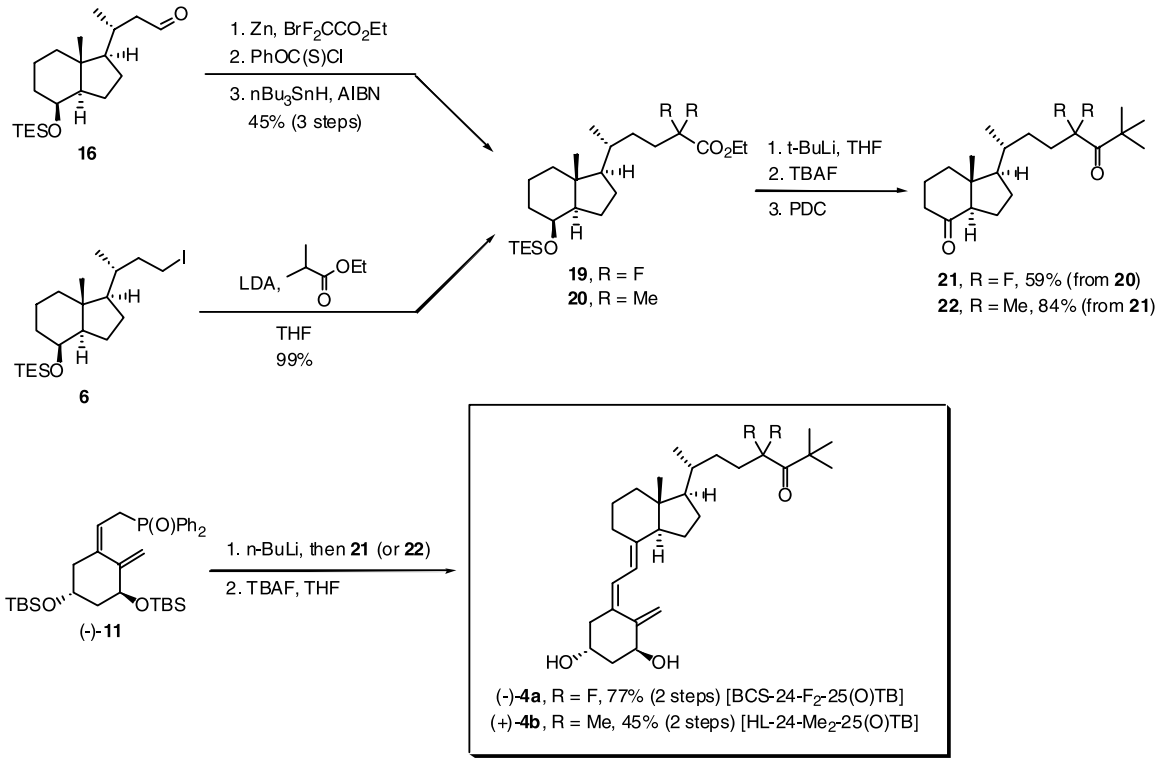
2.2. Biology

Our standard in vitro murine keratinocyte assay[12] indicates that the 25-oxo analogs **2a** and **2b** as well as the 24-oxo analogs **3a** and **3b** are much more antiproliferative than natural calcitriol (**1**) as is especially notable at physiologically relevant low nanomolar concentrations (Fig. 1). Noteworthy is that analog **3a**, differing only slightly from natural calcitriol (**1**) by being a 24-ketone and by having a methyl group in place of the classical 25-OH group, is desirably much more antiproliferative than calcitriol (**1**). Noteworthy also for SAR generalizations is that there is little observed difference in antiproliferative activity between the pair of saturated versus α,β-unsaturated ketones **2a** versus **2b**. 24,24-Difluorinated analog **4a** and 24,24-dimethylated analog **4b**, however, are much less antiproliferative than calcitriol (**1**) and than the corresponding 24-unsubstituted analog **2a** (data not shown); thus, both electron-withdrawing groups (i.e., F) and a sterically hindered 25-carbonyl as in analog **4b** diminish antiproliferative activity considerably.

Our standard in vivo mouse urine assay[12] shows that at least 10 times higher doses of the saturated ketone analogs **2a** and **3a** are required to cause the same level of urinary calcium excretion as the natural hormone calcitriol (**1**, Fig. 2). When dosed at the same level as calcitriol (**1**), ketone **2a** was much less calcemic than calcitriol (**1**). Introduction of a conjugated C=C double bond as in



Scheme 3.



Scheme 4.

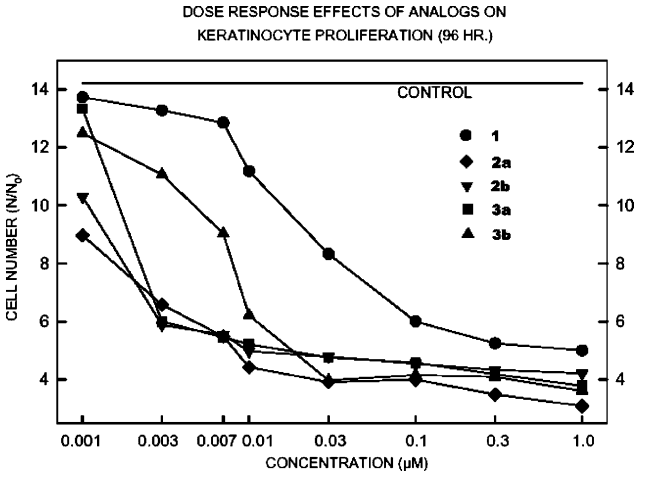


Figure 1. Dose–response effects of analogs on keratinocyte proliferation (96 h).

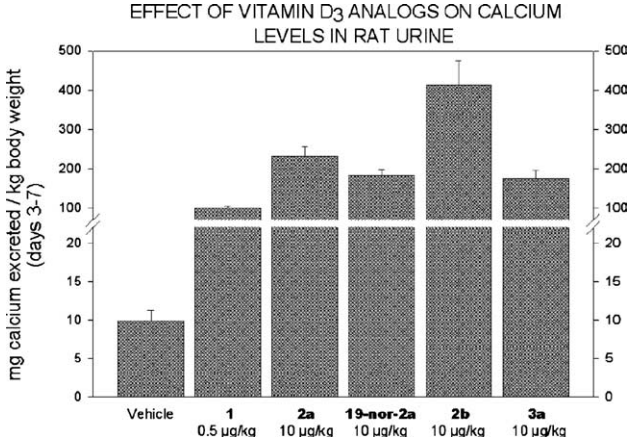


Figure 2. Effect of vitamin D_3 analogs on calcium levels in rat urine.

enone **2b** does not alter calcemic activity very much (Fig. 2). Surprisingly, based on DeLuca's observations that 19-nor analogs usually are much less calcemic than their 19-methylene versions,[7,13] the 19-nor version of 25-ketone **2a** (i.e., 19-nor-**2a**) does not have significantly less calcemic activity than its 19-methylene counterpart **2a**.

3. Conclusions

Two unexpected observations arise from this work: (1) simply replacing the natural 25-OH group in calcitriol (**1**) by a $-CH_3$ group and incorporating a 24-ketone functionality produces analog **3a** that is substantially more antiproliferative in vitro than the natural hormone even at physiologically relevant nanomolar concentrations; and (2) simply removing the one carbon exocyclic 19-methylene group produces analog 19-nor-**2a** that is not significantly less calcemic than 19-methylene analog **2a**. These important SAR generalizations may help design even more potent and safe new analogs of calcitriol (**1**).

4. Experimental

Unless otherwise noted, reactions were run in flame-dried round-bottomed flasks under an atmosphere of ultra-high-purity (UHP) argon. Diethyl ether (ether) and tetrahydrofuran (THF) were distilled from sodium

benzophenone ketyl prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. All other compounds were purchased from Aldrich Chemical Co. and used without further purification. Analytical thin-layer chromatography (TLC) was conducted with silica gel 60 F_{254} plates (250 μm thickness; Merck). Column chromatography was performed using short path silica gel (particle size <230 mesh), flash silica gel (particle size 400–230 mesh), or Florisil (200 mesh). Yields are not optimized. Purity of products was judged to be >95% based on their chromatographic homogeneity. High-performance liquid chromatography (HPLC) was carried out with a Rainin HPLX system equipped with two 25 mL/min preparative pump heads using Rainin Dynamax 10×250 mm (semipreparative) columns packed with 60 Å silica gel (8 μm pore size), either as bare silica or as C-18-bonded silica. Melting points were measured using a Mel-Temp metal-block apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained either on a Varian XL-400 spectrometer, operating at 400 MHz for ^1H and 100 MHz for ^{13}C, or on a Varian XL-500 spectrometer, operating at 500 MHz for ^1H and 125 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrometer. Absorption bands are reported in wave numbers (cm^{-1}). Low- and high-resolution mass spectra (LRMS and HRMS) were obtained with electronic or chemical ionization (EI or CI) either (1) at Johns Hopkins University on a VG Instruments 70-S spectrometer run at 70 eV for EI and run with ammonia (NH_3) as a carrier gas for CI or (2) at the Ohio State University on a Finnigan-MAT CH5, a Finnigan-MAT 731, or a VG Instruments 70-VSE spectrometer run at 70 eV for EI and run with methane (CH_4) for CI.

4.1. *tert*-Butyl C,D-ring ketone 9

A 10-mL round-bottomed flask was charged with diisopropylamine (207 μL, 1.41 mmol distilled over calcium hydride prior to use) and 2 mL of distilled THF. This solution was cooled to −78 °C, and *n*-BuLi (1.0 ml of 1.6 M solution in hexane, 1.37 mmol) was added via syringe. Pinacolone (174 μL, 1.33 mmol dried over potassium carbonate and activated molecular sieve for 24 h immediately prior to use) was dissolved in 1 mL of distilled THF and cooled to −78 °C at which point it was added to the reaction flask via cannula. The reaction was left to stir for 30 min. Hexamethylphosphoramide (HMPA, 300 μL) was then added via syringe, and the reaction mixture was allowed to stir for an additional 15 min. A solution of iodide **5** (0.09 g, 0.19 mmol) in 2 mL of THF was cooled to −78 °C and added to the reaction mixture via cannula. The reaction mixture was stirred at −78 °C for 2 h and to warm to room temperature slowly. The resulting yellow mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (10% ethyl acetate/hexanes) to give a colorless oil **7** (0.06 g, 80%).

A 15-mL round-bottomed flask was charged with TES-*tert*-butyl ketone **7** (0.06 g, 0.2 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride (TBAF 1 M solution in THF, 440 μL, 0.4 mmol) was added to the reaction flask, and this solution was left to stir at room temperature for 7 h. The resulting mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (20% ethyl acetate/hexanes) to give a colorless oil (34 mg, 80%): $[\alpha]_D^{25}$ +20.4 (*c* 2.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.05 (m, 1H), 2.51–2.35 (m, 2H), 1.98–1.94 (m, 1H), 1.89–1.68 (m, 4H), 1.56–1.29 (m, 11H), 1.12 (s, 9H), 0.91 (s, 3H), 0.86 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.5, 69.3, 56.5, 52.5, 44.2, 41.8, 40.3, 34.9, 33.5, 33.3, 29.9, 27.0, 26.4, 22.5, 18.4, 17.4, 13.5; IR (neat, cm^{-1}) 3516, 2935, 2870, 1703, 1477, 1365, 1067, 990; HRMS *m*/*z* [M+Na] calcd 317.2450 for $C_{19}H_{34}O_2Na^+$, found 317.2436.

A flame-dried 25 mL flask equipped with a magnetic stir bar, and a septum along with argon (Ar) ballon was charged with the alcohol *tert*-butyl ketone (0.035 g, 0.11 mmol) and dissolved in 2 mL freshly distilled CH_2Cl_2. Then, to this solution, PDC (125 mg, 0.32 mmol) was added. The resulting mixture was allowed to stir at room temperature for 8 h. The mixture were filtered with Celite, concentrated, and purified using silica gel chromatography (30% ethyl acetate/hexanes) to give diketone **9** as a colorless oil (31 mg, 90%): $[\alpha]_D^{25}$ +11.5 (*c* 0.01, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.47–2.41 (m, 3H), 2.27–2.19 (m, 2H), 2.10 (d, $J = 12.8$ Hz, 1H), 2.02–1.86 (m, 4H), 1.76–1.16 (m, 6H), 1.18 (s, 9H), 0.92 (d, $J = 6.0$ Hz, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.19, 211.94, 61.88, 56.59, 49.82, 44.18, 40.89, 38.89, 35.11, 33.20, 29.80, 27.37, 26.41, 23.97, 19.02, 18.51, 12.45; IR (neat, cm^{-1}) 2958, 2872, 1708, 1474, 1364, 1223, 1068; HRMS *m*/*z* [M+Na] calcd 315.2294 for $C_{19}H_{32}O_2Na^+$, found 315.2276.

4.2. *tert*-Butyl C,D-ring ketone 10

A 10-mL round-bottomed flask was charged with diisopropylamine (322 μL, 2.29 mmol distilled over calcium hydride prior to use) and 2 mL of distilled THF. This solution was cooled to −78 °C, and *n*-BuLi (1.39 mL of 1.6 M solution in hexane, 2.23 mmol) was added via syringe. Pinacolone (272 μL, 2.17 mmol dried over potassium carbonate and activated molecular sieve for 24 h immediately prior to use) was dissolved in 1 mL of distilled THF and cooled to −78 °C at which point it was added to the reaction flask via cannula. The reaction was left to stir for 30 min. Hexamethylphosphoramide (HMPA, 300 μL) was then added via syringe, and the reaction mixture was allowed to stir for an additional 15 min. A solution of iodide **6** (140 mg, 0.31 mmol) in 2 mL of THF was cooled to −78 °C and added to the reaction mixture via cannula. The reaction mixture was stirred at −78 °C for 2 h and to warm to room temperature slowly. The resulting yellow mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and

purified using silica gel column chromatography (4% ethyl acetate/hexanes) to give **8** as a colorless oil (130 mg, 97%).

A 15-mL round-bottomed flask was charged with TES-*tert*-butyl ketone **8** (130 mg, 0.30 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride (TBAF, 1 M solution in THF, 369 μL, 0.36 mmol) was added to the reaction flask, and this solution was left to stir at room temperature for 7 h. The resulting mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (10% ethyl acetate/hexanes) to give a colorless oil (80 mg, 84%): $[\alpha]_D^{25}$ +20.8 (*c* 0.65, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.04 (m, 1H), 2.42–2.39 (m, 2H), 1.97–1.94 (m, 1H), 1.84–1.76 (m, 3H), 1.59–1.15 (m, 8H), 1.10 (s, 9H), 0.90 (s, 3H), 0.89 (d, $J = 7.6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.1, 66.3, 56.3, 52.5, 44.0, 41.8, 40.3, 36.8, 35.3, 35.1, 33.5, 27.1, 26.3, 22.4, 20.2, 18.4, 17.3, 13.5; IR (neat, cm^{-1}) 3358, 2932, 2872, 1701, 1365, 1152, 1023; HRMS *m/z* [M+Na] calcd 331.2607 for $C_{20}H_{36}O_2Na^+$, found 331.2590.

A flame-dried 25-mL flask equipped with a magnetic stir bar, and a septum along with an Ar ballon was charged with the alcohol *tert*-butyl ketone (80 mg, 0.25 mmol) and dissolved in 2 mL freshly distilled CH_2Cl_2. Then, to this solution, PDC (273 mg, 0.72 mmol) was added. The resulting mixture was allowed to stir at room temperature for 8 h. The mixture were filtered with Celite, concentrated, and purified using silica gel chromatography (10% ethyl acetate/hexanes) to give diketone **10** as a colorless oil (79 mg, 99%): $[\alpha]_D^{25}$ +7.6 (*c* 0.75, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 2.44 (m, 3H), 2.29–2.16 (m, 2H), 2.13–2.08 (m, 1H), 2.03–1.80 (m, 2H), 1.77–1.25 (m, 12H), 1.12 (s, 9H), 0.97 (d, $J = 6.4$ Hz, 3H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.0, 212.1, 62.0, 56.3, 49.9, 44.1, 40.9, 38.9, 36.6, 35.4, 35.3, 27.4, 26.4, 24.0, 20.1, 19.0, 18.6, 12.4; IR (neat, cm^{-1}) 2958, 2873, 1711, 1478, 1367, 1073; HRMS *m/z* [M+Na] calcd 329.2451 for $C_{20}H_{34}O_2Na^+$, found 329.2469.

4.3. 25(O) Analogs (+)-2a and (+)-2b′

Racemic phosphine oxide (±)-**11** and enantiomerically pure C,D-ring ketone (+)-**10**, each was separately azeotropically dried with anhydrous benzene (4× 1 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use. A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with racemic phosphine oxide (±)-**11** (70 mg, 0.12 mmol) which was dissolved in 2 mL freshly distilled THF. The flask was cooled down to −78 °C in a dry ice bath. To this solution, *n*-BuLi (82 μL, 0.12 mmol, 1.6 M solution in hexanes) was added dropwise over several minutes, during which time a deep red color was developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL recovery flask equipped with a magnetic stir bar, and a septum along with an Ar balloon was charged with C,D-ring ketone **10** (33 mg, 0.1 mmol), dissolved in 1 mL freshly distilled THF, and cooled down to −78 °C in a dry ice bath. The solution of C,D-ring ketone was gently transferred into the flask containing the phosphine oxide anion at −78 °C over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at −78 °C for 8 h, during that time it was visually checked. On observation of the light yellow color, the reaction was quenched at −78 °C by adding 3 mL of pH 7 buffer and allowed to come to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate and extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL) and brine solution, dried over $MgSO_4$, filtered, and purified using silica gel chromatography (5% ethyl acetate/hexanes) to give a colorless oil (40 mg, 55%).

A 15-mL round-bottomed flask was charged with the coupled compound (20 mg, 0.02 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride (TBAF, 1 M solution in THF, 148 μL, 0.1 mmol) was added to the reaction flask, and this solution was left to stir at room temperature for 7 h. The resulting mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (25% ethyl acetate/hexanes) to give **2a** and **2a′** (11 mg, 83%) as a mixture of diastereomers. The diastereomeric mixture was separated by an HPLC using Chiral OD [semipreparative (1× 25 cm), flow rate = 2.5 mL/min] eluted with 2.5% isopropyl alcohol in hexanes to afford 4 mg of **2a** (1α , 3β) and 1.3 mg of **2a′** (1β , 3α) in 55 and 17% yield. The retention time for **2a** is 103.15 min and **2a′** is 121.28 min. Data for **2a** (1α, 3β): $[\alpha]_D^{25}$ +20.3 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, $J = 11.2$ Hz, 1H), 5.99 (d, $J = 11.2$ Hz, 1H), 5.31 (s, 1H), 4.98 (s, 1H), 4.41 (m, 1H), 4.21 (m, 1H), 2.80 (dd, $J = 12.0$, 4.0 Hz, 1H), 2.58 (dd, $J = 12.0$, 2.8 Hz, 1H), 2.42 (m, 2H), 2.29 (dd, $J = 13.6$, 6.4 Hz, 1H), 2.03–1.82 (m, 5H), 1.66–1.62 (m, 2H), 1.53–1.51 (m, 3H), 1.48–1.23 (m, 10H), 1.11 (s, 9H), 0.92 (d, $J = 6.4$ Hz, 3H), 0.515 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.2, 147.6, 143.3, 132.8, 125.0, 116.9, 111.8, 70.8, 66.9, 56.3, 56.2, 45.9, 45.3, 44.1, 42.8, 40.4, 36.9, 36.0, 35.5, 29.1, 27.6, 26.4, 23.6, 22.2, 20.4, 18.8, 12.0; IR (neat, cm^{-1}) 3374, 2949, 2360, 1705, 1464, 1366, 1054; HRMS *m/z* [M+Na] calcd 465.3339 for $C_{29}H_{46}O_3Na^+$, found 465.3310. Data for **2a′** (1β, 3α): $[\alpha]_D^{25}$ +3.9 (*c* 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, $J = 11.2$ Hz, 1H), 6.05 (d, $J = 11.2$ Hz, 1H), 5.32 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.21 (m, 1H), 2.82 (dd, $J = 12.0$, 3.6 Hz, 1H), 2.61 (dd, $J = 13.2$, 4.0 Hz, 1H), 2.44 (m, 3H), 2.30 (dd, $J = 13.2$, 7.6 Hz, 1H), 2.04–1.82 (m, 5H), 1.68–1.63 (m, 2H), 1.55–1.49 (m, 3H), 1.47–1.25 (m, 10H), 1.31 (s, 9H), 0.94 (d, $J = 6.4$ Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.2, 247.2, 143.3, 132.6, 125.0, 117.0, 112.6, 94.4, 71.4, 66.8, 56.3, 56.2, 45.9, 45.5, 42.8, 40.4, 36.9, 36.0, 35.5, 29.0, 27.6, 26.4, 23.6, 22.3, 20.4, 18.8, 12.0; IR (neat, cm^{-1}) 3368, 2951, 1705, 1465, 1054; HRMS *m/z* [M+Na] calcd 465.3339 for $C_{29}H_{46}O_3Na^+$, found 465.3332.

4.4. 19-nor-25(O) Analog (+)-2a

19-nor Phosphine oxide (+)-**11** and enantiomerically pure C,D-ring ketone (+)-**10**, each was separately azeotropically dried with anhydrous benzene (4× 1 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use. A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with phosphine oxide (+)-**11** (60 mg, 0.12 mmol) which was dissolved in 2 mL freshly distilled THF. The flask was cooled down to −78 °C in a dry ice bath. To this solution was added *n*-BuLi (81 μL, 0.12 mmol, 1.42 M solution in hexanes) dropwise over several minutes, during which time a deep red color was developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with C,D-ring ketone (+)-**10** (27 mg, 0.08 mmol), dissolved in 1 mL freshly distilled THF, and cooled down to −78 °C in a dry ice bath. The solution of C,D-ring ketone was gently transferred into the flask containing the phosphine oxide anion at −78 °C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at −78 °C for 8 h, during which time it was visually checked. On observation of the light yellow color, the reaction was quenched at −78 °C by adding 3 mL of pH 7 buffer and allowed to come to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate and extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL) and brine solution, dried over $MgSO_4$, filtered, and purified using silica gel chromatography (2% ethyl acetate/hexanes) to give a colorless oil (8 mg, 15%).

A 15-mL round-bottomed flask was charged with the coupled compound (8 mg, 0.02 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride (TBAF, 1 M solution in THF, 148 μL, 0.1 mmol) was added to the reaction flask, and this solution was left to stir at room temperature for 7 h. The resulting mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (25% ethyl acetate/hexanes) to give 19- nor-**2a** (4 mg, 85%): $[\alpha]_D^{25}$ +15.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (d, J = 11.6 Hz, 1H), 5.85 (d, J = 11.2 Hz, 1H), 4.41 (m, 1H), 4.05 (m, 1H), 2.81–2.71 (m, 2H), 2.50–2.18 (m, 5H), 2.11–1.22 (m, 18H), 1.13 (s, 9H), 0.94 (d, J = 6.8 Hz, 3H), 0.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.2, 143.2, 131.0, 123.9, 115.2, 67.4, 67.2, 56.3, 56.2, 45.8, 44.7, 42.2, 40.4, 37.2, 36.9, 36.0, 35.5, 29.7, 28.9, 27.6, 26.4, 23.5, 22.2, 20.4, 18.8, 12.0; IR (neat, cm^{-1}) 3500, 2926, 1706, 1364, 1049; HRMS *m/z* [M+Na] calcd 453.3339 for $C_{28}H_{46}O_3Na^+$, found 453.3351; UV (CH_2Cl_2) λ_{max} = 255 nm (ε = 29,530).

4.5. 24(O)TB analog (+)-3a

Enantiomerically pure A-ring phosphine oxide (−)-**11** and C,D-ring ketone (+)-**9**, each was separately azeotropically dried with anhydrous benzene (4× 1 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use. A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with enantiomerically pure A-ring phosphine oxide (−)-**11** (50 mg, 0.08 mmol) which was dissolved in 2 mL freshly distilled THF. The flask was cooled down to −78 °C in a dry ice bath. To this solution, *n*-BuLi (48 μL, 0.08 mmol, 1.6 M solution in hexanes) was added dropwise over several minutes, during which time a deep red color was developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL recovery flask equipped with a magnetic stir bar, and a septum along with an Ar balloon was charged with enantiomerically pure C,D-ring ketone (+)-**9** (22 mg, 0.075 mmol), dissolved in 1 mL freshly distilled THF, and cooled down to −78 °C in an dry ice bath. The solution of C,D-ring ketone was gently transferred into the flask containing the phosphine oxide anion at −78 °C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at −78 °C for 9 h, during that time it was visually checked. On observation of the light yellow color, the reaction was quenched at −78 °C by adding 3 mL of pH 7 buffer and allowed to come to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate and extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL) and brine solution, dried over $MgSO_4$, filtered, and purified using silica gel chromatography (5% ethyl acetate/hexanes) to give a colorless oil coupled product (13 mg, 35%).

A 15-mL round-bottomed flask was charged with the coupled product (13 mg, 0.02 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride (TBAF 1 M solution in THF, 59 μL, 0.06 mmol) was added to the reaction flask, and this solution was left to stir at room temperature for 7 h. The resulting mixture quenched with 2 mL of water, extracted with ethyl acetate (3× 25 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (25% ethyl acetate/hexanes) to give 24(*O*)TB **3** (7 mg, 82%) as a colorless oil. The product was separated by an HPLC using Chiral OD [semipreparative (1× 25 cm), t_R = 38.5 min] eluted with 7% IPA in hexanes to afford 4 mg of 24(*O*)TB **3**: $[\alpha]_D^{25}$ +29.6 (*c* 3.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (d, J = 11.2 Hz, 1H), 5.99 (d, J = 11.2 Hz, 1H), 5.32 (s, 1H), 4.99 (s, 1H), 4.42 (m, 1H), 4.22 (m, 1H), 2.81 (dd, J = 12.0, 4.0 Hz, 1H), 2.59 (dd, J = 12.0, 2.8 Hz, 1H), 2.52–2.28 (m, 5H), 2.20–1.20 (m, 16H), 1.13 (s, 9H), 0.91 (d, J = 6.4 Hz, 3H), 0.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.5, 147.5, 143.1, 132.9, 124.9, 117.0, 111.8, 70.8, 66.8, 56.4, 56.2, 45.8, 45.2, 44.2, 42.8, 40.4, 35.7, 33.2, 30.2, 29.0, 27.5, 26.4, 23.5, 22.2, 18.6, 14.6, 12.0; IR (neat, cm^{-1}) 3356, 2949, 2872, 1703, 1465, 1364, 1054, 754; HRMS *m/z* [M+Na] calcd 451.3182 for $C_{28}H_{44}O_3Na^+$, found 451.3178.

4.6. Cyano-C,D-ring ketone 13

A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, and a septum along with an Ar balloon was charged with C-8 alcohol (50 mg, 0.22 mmol) dissolved in 2.2 mL freshly distilled CH_2Cl_2. To this solution, PDC (170 mg, 0.45 mmol) and 0.22 g of oven-dried Celite were added in one portion at room temperature. The resulting mixture was allowed to stir at room temperature for about 12 h. The mixture was directly purified by column chromatography eluted with first 20% ethyl acetate in hexanes to afford 43 mg of ketone **13** in 83% yield: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.52 (dd, J = 7.6 Hz, 1H), 2.41–2.21 (m, 4H), 2.12–2.01 (m, 2H), 1.99–1.74 (m, 4H), 1.69–1.54 (m, 2H), 1.37–1.25 (m, 1H), 1.2 (d, J = 6.8 Hz, 3H), 0.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.1, 118.4, 61.5, 54.9, 49.5, 40.7, 38.4, 33.1, 27.3, 24.6, 23.7, 19.2, 18.9, 12.5; HRMS m/z [M+Na] calcd 242.1515 for $C_{14}H_{21}NO$, found 242.1519.

4.7. 23-Enone analogs (+)-2b and 2b′

Phosphine oxide (±)-**11** and C,D-ring ketone **29**, each was separately azeotropically dried with anhydrous benzene (4× 1 mL) on a rotary evaporator and held under vacuum (ca. 0.1 mmHg) for at least 48 h prior to use. A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with phosphine oxide (±)-**11** (60 mg, 0.1 mmol) and dissolved in ca 1.2 mL freshly distilled THF to give ca. 0.1 M solution, and the flask was cooled down to −78 °C in a dry ice bath. To this solution, *n*-BuLi (70 μL, 0.11 mmol, 1.6 M solution in hexanes) was added dropwise over several minutes, during which time a deep red color was developed and persisted. This mixture was allowed to stir at −78 °C for an additional 10 min. Meanwhile, a flame-dried 10-mL recovery flask equipped with a magnetic stir bar, and a septum along with an Ar balloon was charged with C,D-ring ketone **13** (15 mg, 0.068 mmol), dissolved in 0.5 mL freshly distilled THF, and cooled down to −78 °C in a dry ice bath. The solution of C,D-ring ketone **13** was transferred dropwise into the flask containing the phosphine oxide anion at −78 °C via cannula over several minutes. After the addition was complete, the deep red color persisted and the mixture was allowed to stir at −78 °C for 2 h, during that time it was visually checked. On observation of the light yellow color, the reaction was quenched at −78 °C by adding of 3 mL of pH 7 buffer and allowed to come to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate and extracted with ethyl acetate (4× 25 mL). The combined extracts were washed with water (25 mL) and brine solution (25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product that was purified by column chromatography eluted with 25% ethyl acetate in hexanes in the presence of 1% triethylamine to afford 34 mg of the coupled product in 85% yield in a 3.8:1 ratio as determined by ^{1}H NMR.

This coupled product (30 mg, 0.051 mmol) was charged into 5-mL round-bottomed flask equipped with a magnetic stir bar, and a septum along an Ar balloon and dissolved in 1 mL anhydrous dichloromethane to give ca. 0.05 M solution. Then the flask was cooled down to −78 °C. To this well-stirred solution, DIBAL (0.077 mmol, 51.6 mL, 1.5 M solution in toluene) was added via syringe at this temperature and the mixture was then allowed to stir at −78 °C for 1 h. This reaction mixture was diluted with ether (25 mL), and 1 N solution of HCl (ca. 1 mL) was added and stirred for a few minutes. The reaction mixture was then rinsed into a separatory funnel with ethyl acetate and was extracted with ethyl acetate (4× 10 mL). The combined extracts were washed with water (10 mL) and brine solution (10 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product that was purified by flash column chromatography eluted with 5% ethyl acetate in hexanes in the presence of 1% triethylamine affording 25 mg of 23-aldehyde **14** in 83% yield.

A flame-dried 10-mL recovery flask equipped with a magnetic stir bar, and a septum along with an Ar balloon was charged with KOtBu (3.5 mg, 0.031 mmol) and 0.5 mL freshly distilled THF. Then, the flask was cooled down to −78 °C in a dry ice bath. To this solution, dimethylphosphonate **15** (6.4 mg, 0.031 mmol) was added as a solution in 0.5 mL freshly distilled THF. After 30 min, 0.5 mL of THF solution of 23-aldehyde **14** (15 mg, 0.026 mmol) was transferred into the flask via cannula over several minutes at −78 °C. After the addition was complete, the mixture was gradually warmed up to room temperature and then stirred for about 4 h. The reaction was quenched by adding 2 mL distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 10 mL). The combined extracts were washed with water (10 mL), and brine solution (10 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product that was purified by flash column chromatography eluted with 5% ethyl acetate in hexanes to afford 12 mg of *trans*-olefin as determined by ^{1}H NMR in 70% yield.

This product (12 mg, 0.018 mmol) was charged into a 5 mL argon purged polypropylene vial equipped with a magnetic stir bar, a septum along with a cap and dissolved in 1.0 mL acetonitrile to give ca. 0.02 M solution. To this well-stirred solution, HF (1.8 mmol, 49% aqueous solution) was added via syringe at room temperature and the mixture was then allowed to stir at room temperature for 2 h. TLC showed the completion of the reaction. This reaction mixture was diluted with ether (25 mL) and saturated solution of $NaHCO_3$ was added until no more carbon dioxide was liberated. The reaction mixture was then rinsed into a separatory funnel with ethyl acetate and was extracted with ethyl acetate (4× 10 mL). The combined extracts were washed with water (10 mL) and brine solution (10 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product as a mixture of diastereomers in 4:1 ratio (as determined by ^{1}H NMR of the crude reaction mixture). The crude was purified by column chromatography eluted with 100% ethyl acetate in the presence of 1%

TEA to afford 5.2 mg of a diastereomeric mixture of **2b** and **2b′** in 66% yield. This analog **2b** was purified by an HPLC [semipreparative (1× 25 cm) Chiracel OD, t_R = 48.2 min] eluted with 5% isopropyl alcohol in hexanes to afford 2.2 mg of **2b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.95–6.88 (m, 1H), 6.49 (d, J = 15.2 Hz, 1H), 6.37 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.6 Hz, 1H), 5.33–5.32 (m, 1H), 5.00–4.99 (m, 1H), 4.45–4.01 (m, 1H), 4.25–4.22 (m, 1H), 2.84 (dd, J = 12.0, 4.4 Hz, 1H), 2.60 (dd, J = 13.2, 5.0 Hz, 1H), 2.34–2.29 (m, 2H), 2.07–1.89 (m, 6H), 1.68–1.49 (m, 17H), 1.35–1.25 (m, 4H), 0.95 (d, J = 6.4 Hz, 3H), 0.55 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.3, 147.6, 146.2, 142.9, 132.9, 125.7, 124.9, 117.1, 111.8, 70.8, 66.8, 56.2, 56.0, 45.9, 45.2, 42.8, 42.7, 40.3, 39.3, 36.0, 29.0, 27.6, 26.2, 23.5, 22.2, 19.1, 12.0; IR (neat, cm^{-1}) 3369, 2949, 2872, 1684, 1619, 1437, 1366, 1055, 908; HRMS *m/z* [M+Na] calcd 463.3183 for $C_{30}H_{45}NO_3Na^+$, found 463.3193; UV (MeOH) λ_{max} = 265 nm (ε = 15,864).

4.8. 22-Ene-24 ketone silyl ether 17

To a solution of the phosphate **15** (80 mg, 0.38 mmol) in THF (5 mL), potassium *tert*-butoxide (43 mg, 0.38 mg) was added at 0 °C. After stirring for 1 h at 0 °C, a solution of the aldehyde (+)-**16** (60 mg, 0.18 mmol) in THF (2 mL) was added via cannula at rt. Then, the mixture was stirred for 4 days at rt. The resulting mixture was quenched with water (3 mL), extracted with EtOAc (3× 25 mL), dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography with EtOAc/hexanes (1:15) as eluent to afford 50 mg (68%) of the desired ketone **17** as a colorless oil: $[\alpha]_D^{25}$ +73.4 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (dd, J = 15.2, 9.2 Hz, 1H), 6.39 (dd, J = 15.2, 0.8 Hz, 1H), 4.03 (m, 1H), 2.24 (m, 1H), 1.93 (dt, J = 12.4, 2.8 Hz, 1H), 1.82 (m, 1H), 1.50–1.69 (m, 4H), 1.13–1.41 (m, 6H), 1.14 (s, 9H), 1.05 (d, J = 6.4 Hz, 3H), 0.94 (s, 3H), 0.935 (t, J = 8.0 Hz, 9H), 0.54 (q, J = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.8, 152.9, 121.8, 69.3, 55.5, 52.9, 42.8, 42.4, 40.6, 39.7, 34.6, 27.4, 26.2, 23.0, 19.2, 17.7, 13.8, 6.9, 4.9; IR (neat, cm^{-1}) 2953, 2875, 1725, 1690, 1623, 1457, 1366, 1234, 1166, 1081, 1018, 725; HRMS *m/z* [M+Na] calcd 429.3159 for $C_{25}H_{46}O_2SiNa^+$, found 429.3161.

4.9. 22-Ene-24 ketone C,D-ring ketone 18

To a solution of silyl ether **17** (46 mg, 0.11 mmol) in THF (3 mL), 0.35 mL (0.35 mmol) of a 1.0 M solution of TBAF in THF was added at rt, and then it was stirred overnight at rt. The reaction mixture was quenched with water (2 mL), extracted with EtOAc (3×, 20), washed with brine, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography with EtOAc/hexanes (1:3) as eluent to give 12 mg (37%) of the desired alcohol as a colorless oil.

To a solution of the alcohol (12 mg, 0.041 mmol) in CH_2Cl_2 (4 mL), 120 mg of oven-dried Celite and PDC (120 mg, 0.33 mmol) was added at room temperature. The mixture solution was stirred overnight and then passed through a 2 cm pad of flash silica gel and washed with EtOAc. The filtrate was concentrated and subjected to column chromatography with EtOAc/hexanes (1:4) as eluent to give 11 mg (90%) of the desired C,D-ring ketone **18** as a colorless oil: $[\alpha]_D^{25}$ +30.8 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, CDCl3) δ 6.74 (dd, J = 15.2, 9.2 Hz, 1H), 6.42 (dd, J = 15.2, 8.0 Hz, 1H), 2.46 (dd, J = 11.2, 7.6 Hz, 1H), 2.18–2.32 (m, 3H), 2.00–2.12 (m, 2H), 1.91 (m, 1H), 1.46–1.78 (m, 5H), 1.27 (m, 1H), 1.14 (s, 9H), 1.12 (d, J = 6.4 Hz, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.5, 204.6, 151.5, 122.3, 61.7, 55.3, 49.9, 42.9, 40.9, 39.7, 38.8, 27.5, 26.2, 24.0, 19.5, 19.1, 12.7; IR (neat, cm^{-1}) 2961, 2872, 1713, 1688, 1622, 1477, 1366, 1233, 1076, 989, 950, 860; HRMS *m/z* [M+Na] calcd 313.2138 for $C_{19}H_{30}O_2Na^+$, found 313.2132.

4.10. 22-Ene-24(O)TB (+)-3b

A solution of 50 mg (0.086 mmol) of enantiomerically pure phosphine oxide (−)-**11** in 1.5 mL of anhydrous THF was cooled to −78 °C and treated with 54 μL (0.086 mmol, 1.6 M in hexanes) of *n*-BuLi under argon atmosphere. The mixture turned deep reddish color and was stirred for 15 min at −78 °C. To the solution, a precooled (−78 °C) solution of 10 mg (0.034 mmol) of the enantiomerically pure C,D-ring ketone (+)-**18** in 1.5 mL of anhydrous THF via cannula was added dropwise. The reaction kept going until the reddish-orange color faded to yellow (about 3 h). The reaction was quenched by adding 1.0 mL of pH 7 buffer at −78 °C, and then warmed to room temperature, extracted with EtOAc (3× 20 mL), washed with brine, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography with EtOAc/hexanes (1:10) as eluent to afford 4 mg (18%) of the coupled product as a colorless oil.

The coupled product (4 mg, 0.0060 mmol) was dissolved in 2 mL of anhydrous EtOH, and to the solution, 50 μL of 49% aq HF was added. The resulting mixture was stirred 2 h at room temperature, and then quenched with 5 mL of satd $NaHCO_3$ solution. The solution was stirred for 10 min, and then extracted with EtOAc (3× 20 ml), washed with brine, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography with EtOAc as eluent to give 2 mg (93%) of the desired product **3b** as a colorless oil: $[\alpha]_D^{25}$ +65.0 (c 0.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (dd, J = 15.2, 9.2 Hz, 1H), 6.41 (d, J = 15.2 Hz, 1H), 6.37 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 5.32 (m, 1H), 4.99 (m, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 2.83 (dd, J = 11.6, 3.2 Hz, 1H), 2.60 (dd, J = 13.2, 3.2 Hz, 1H), 2.20–2.34 (m, 2H), 1.89–2.04 (m, 4H), 1.43–1.76 (m, 6H), 1.14–1.41 (m, 5H), 1.15 (s, 9H), 1.09 (d, J = 6.8 Hz, 3H), 0.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.1, 152.4, 147.6, 142.6, 133.1, 124.9, 122.1, 117.2, 111.8, 70.8, 66.8, 56.1, 55.3, 46.1, 45.3, 42.8, 40.3, 40.2, 29.7, 29.0, 27.4, 26.2, 23.5, 22.3, 19.6, 12.3; IR (neat, cm^{-1}) 3401, 2955, 2926, 2872, 1684, 1653, 1617, 1558, 1546, 1507, 1457, 1079.

4.11. Difluoro ethyl ester 19

A suspension of activated zinc dust (98.5 mg, 1.51 mmol) and ethyl bromodifluoroacetate (0.19 mL, 1.51 mmol) in THF (5 mL) was refluxed for 20 min and then cooled to 0 °C. To this, the solution of the aldehyde (+)-**16** (100 mg, 0.30 mmol) in THF (5 mL) was added. The reaction mixture was warmed to room temperature, followed by refluxing for 20 min, and then cooled to room temperature. The reaction mixture was poured into 1 M $KHSO_4$ and extracted with EtOAc (3× 20 mL), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (10% EtOAc/hexanes) to give 84.5 mg (62%) of a 1:1 mixture of diastereomers of the desired alcohol as a colorless oil.

To a solution of ethyl ester (84 mg, 0.18 mmol) and pyridine (0.066 mL, 0.82 mmol) in CH_2Cl_2 (5 mL), phenyl chlorothionoformate (0.053 mL, 0.38 mmol) was added. After being stirred at room temperature for 20 h, the reaction mixture was diluted with water, extracted with ether (3× 20 mL), washed with satd $NaHCO_3$ solution and brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (5% EtOAc/hexanes) to give 97.8 mg (90%) of the desired carbonate as diastereomeric mixtures.

To the solution of the resulting phenylthianocarbonate (97.5 mg, 0.16 mmol) in anhydrous benzene (10 mL), 2,2′-azobisisobutyronitrile (AIBN, 5 mg) and tributyltin hydride (0.066 mL, 0.24 mmol) were added at room temperature. After being refluxed for 3 h, the mixture was cooled to 0 °C, diluted with water, extracted with EtOAc (3× 20 mL), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (3% EtOAc/hexanes) to give 58.2 mg (80%) of the desired difluoro ester **19** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 (q, J = 7.2 Hz, 2H), 4.03 (s, 1H), 2.14–2.06 (m, 1H), 1.98–1.89 (m, 2H), 1.85–1.75 (m, 2H), 1.69–1.64 (m, 1H), 1.61–1.53 (m, 2H), 1.49–1.43 (m, 1H), 1.38–1.34 (m, 2H), 1.35 (t, J = 7.2 Hz, 3H), 1.26–1.17 (m, 3H), 1.13–0.99 (m, 2H), 0.94 (t, J = 8.0 Hz, 9H), 0.91 (d, J = 6.0 Hz, 3H), 0.90 (s, 3H), 0.55 (q, J = 8.0 Hz, 6H).

4.12. Difluoro-C,D-ring diketone 21

To a solution of ethyl ester **19** (54 mg, 0.12 mmol) in THF (5 mL) was added 125.1 μL of *tert*-BuLi (1.43 M in pentane, 0.18 mmol) at −78 °C. After being stirred for 3 h, the reaction mixture was warmed up to room temperature, diluted with water, extracted with ether (3× 20 mL), washed with satd $NaHCO_3$ solution and brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (hexanes only) to give 41.6 mg (75%) of the desired ketone as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (d, J = 2.4 Hz, 1H), 1.95–1.90 (m, 1H), 1.87–1.74 (m, 2H), 1.69–1.64 (m, 1H), 1.57–1.52 (m, 2H), 1.49–1.43 (m, 2H), 1.39–1.31 (m, 3H), 1.26 (s, 9H), 1.24–1.20 (m, 2H), 1.12–0.99 (m, 2H), 0.94 (t, J = 8.0 Hz, 9H), 0.89 (d, J = 6.0 Hz, 3H), 0.89 (s, 3H), 0.55 (q, J = 8.0 Hz, 6H).

To a solution of ketone (30 mg, 0.065 mmol) in THF (5.0 mL), 0.20 mL (0.20 mmol) of a 1.0 M solution of TBAF in THF was added, and then it was stirred at 0 °C for 1 h and stirred overnight at room temperature. The reaction mixture was quenched with water (5 mL), extracted with EtOAc (3× 20 mL), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (25% EtOAc/hexanes) to give 21.0 mg (93%) of alcohol as a colorless oil.

To a solution of the C,D-ring alcohol (14.1 mg, 0.041 mmol) in CH_2Cl_2 (5 mL) were added 50 mg of oven-dried Celite and PDC (46.2 mg, 0.12 mmol) at room temperature. The reaction mixture was stirred overnight and then passed through a 2 cm pad of flash silica gel and washed with EtOAc. The filtrate was concentrated and purified by column chromatography (20% EtOAc/hexanes) to give 13.0 mg (93%) of the desired C,D-ring diketone **21** as a colorless oil: $[\alpha]_D^{25}$ +1.30 (c 1.01, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.45 (dd, J = 11.6, 7.6 Hz, 1H), 2.30–2.21 (m, 2H), 2.11–2.07 (m, 2H), 2.05–1.97 (m, 1H), 1.96–1.84 (m, 3H), 1.76–1.71 (m, 1H), 1.61–1.42 (m, 5H), 1.33–1.21 (m, 2H), 1.25 (s, 9H), 0.97 (d, J = 6.0 Hz, 3H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.83, 205.23 (J = 15.5 Hz), 120.74 (J = 253.1 Hz), 61.87, 55.99, 49.81, 43.34, 40.90, 38.89, 34.91, 30.54 (J = 22.7 Hz), 27.30, 27.04 (J = 4.1 Hz), 26.07 (J = 2.3 Hz), 23.99, 19.01, 18.41, 12.47; IR (neat, cm^{-1}) 2954, 2860, 1719, 1460, 1366, 1307, 1231, 1196, 1043, 1008, 967, 943, 914, 843, 773, 737; HRMS m/z [M+Na] calcd 365.2262 for $C_{20}H_{32}F_2O_2Na^+$, found 365.2252.

4.13. 24-F_2-25(O)TB analog (−)-4a

To a solution of 50.0 mg (0.086 mmol) of enantiomerically pure A-ring phosphine oxide (−)-**11** in 2.0 mL of anhydrous THF, 53.6 μL (0.086 mmol, 1.6 M in hexanes) of *n*-BuLi was added at −78 °C, and then the reddish solution was stirred for 15 min at −78 °C. To the solution, a precooled (−78 °C) solution of enantiomerically pure C,D-ring ketone (+)-**21** (8.0 mg, 0.023 mmol) in 1.0 mL of anhydrous THF was added dropwise. The reaction kept going until the reddish-orange color faded to yellow (about 6 h). The reaction was quenched by adding 1.0 mL of pH 7 buffer, and then warmed up to room temperature, extracted with EtOAc (2× 20 mL), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (20% EtOAc/hexanes) to afford 13.3 mg (81%) of the coupled product as a pale yellow oil.

The coupled product (13.2 mg, 0.019 mmol) was dissolved in 5 mL of anhydrous THF, and to this solution, 0.075 mL (0.075 mmol) of a 1.0 M solution of TBAF in THF was added. The reaction was run in darkness overnight, and then extracted with EtOAc (2× 30 mL), washed with brine, dried over $MgSO_4$, concentrated in vacuo, and then purified by column chromatography (5% MeOH/CH_2Cl_2) to give 8.48 mg (95%) of the desired compound **4a** as a colorless oil: $[\alpha]_D^{25}$ −23.3 (c 0.42, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, J = 11.6 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 5.32

(t, J = 1.6 Hz, 1H), 5.00 (t, J = 1.6 Hz, 1H), 4.44–4.41 (m, 1H), 4.24–4.21 (m, 1H), 2.82 (dd, J = 12.8, 4.4 Hz, 1H), 2.60 (dd, J = 13.6, 3.6 Hz, 1H), 3.34–2.28 (m, 1H), 2.07–1.82 (m, 6H), 1.70–1.58 (m, 5H), 1.55–1.42 (m, 5H), 1.31–1.27 (m, 2H), 1.26 (s, 9H) 0.94 (d, J = 6.4 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.33 (J = 29.1 Hz), 147.58, 142.95, 132.96, 124.93, 120.86 (J = 253.6 Hz), 117.09, 111.81, 70.82, 66.84, 56.26, 55.86, 45.85, 45.24, 43.34, 42.82, 40.39, 35.50, 30.64 (J = 22.7 Hz), 29.03, 27.39, 27.13, 26.08, 23.52, 22.19, 18.51, 11.98; IR (neat, cm^{-1}) 3365, 2942, 2860, 1725, 1642, 1454, 1366, 1225, 1049, 961, 908, 796, 749, 737, 596; UV (MeOH) λ_{max} = 252 nm (ε = 3166).

4.14. Ester 20

Lithium diisopropylamide (LDA) solution was prepared by treating diisopropylamine (213.5 mg, 2.11 mmol) in THF (5 mL) at −78 °C with a 1.6 M solution of *n*-BuLi in hexanes (1.3 mL, 2.08 mml). The LDA solution was stirred at −78 °C for 30 min, and then a solution of ethyl isobutyrate (245 mg, 2.11 mmol) in THF (2 mL) was added. After being stirred for 1 h at −78 °C, a solution of iodide (+)-**6** (95 mg, 0.211 mmol) in THF (2 mL) was added. The reaction mixture was stirred at −78 °C for 2 h, then warmed up to room temperature and quenched with the addition of water (5 mL). The reaction mixture was extracted with diethyl ether (4× 15 mL). The organic layer was washed with water and brine, dried over sodium sulfate, concentrated in vacuo, and then purified by column chromatography (5% EtOAc/petroleum ether) to give 91.8 mg of ester **20** (0.209 mmol, 99% yield) as a colorless oil: $[\alpha]_D^{24}$ +41.8 (c 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.104 and 4.102 (q, J = 7.2 Hz, 2H), 4.03–4.02 (m, 1H), 1.93 (dm, J = 12.4 Hz, 1H), 1.83–1.72 (m, 2H), 1.67–1.65 (m, 1H), 1.61–1.50 (m, 5H), 1.43–1.27 (m, 6H), 1.24 (t, J = 7.2 Hz, 3H), 1.19–0.97 (m, 2H), 1.14 (s, 3H), 1.137 (s, 3H), 0.94 (t, J = 8.0 Hz, 9H), 0.88 (s, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.55 (q, J = 8.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.12, 69.38, 60.10, 56.28, 53.07, 42.15, 42.07, 40.74, 36.97, 35.40, 34.65, 30.61, 27.11, 25.25, 25.11, 22.99, 18.63, 17.67, 14.28, 13.46, 6.93, 4.93; IR (neat, cm^{-1}) 2950, 2875, 1731, 1472, 1144, 1025, 742; HRMS m/z [M+Na] calcd 461.3421 for $C_{26}H_{50}O_3SiNa^+$, found 461.3398.

4.15. Diketone 22

To a solution of ester **20** (90 mg, 0.205 mmol) in diethyl ether (4 mL), a solution of *tert*-butyllithium (1.43 M in pentane, 0.15 mL, 0.215 mmol) was added dropwise at −78 °C. After being stirred for 1 h at −78 °C, the reaction mixture was warmed up to room temperature, and then quenched with the addition of water (4 mL). The reaction mixture was extracted with diethyl ether (4× 15 mL). The organic layer was washed with water and brine, dried over sodium sulfate, concentrated in vacuo, and then purified by column chromatography (5% EtOAc/petroleum ether) to give 83.1 mg of *tert*-butyl ketone (0.184 mmol, 90% yield) as a colorless oil: $[\alpha]_D^{24}$ +41.1 (c 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.02–4.01 (m, 1H), 1.93 (dm, J = 12.8 Hz, 1H), 1.83–1.72 (m, 2H), 1.67–1.42 (m, 5H), 1.38–0.99 (m, 7H), 1.37–1.14 (m, 2H), 1.23 (s, 3H), 1.22 (s, 9H), 1.19 (s, 3H), 0.94 (t, J = 8.0 Hz, 9H), 0.88 (s, 3H), 0.87 (d, J = 6.4 Hz, 3H), 0.54 (q, J = 8.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 218.85, 69.39, 56.49, 53.06, 49.48, 45.66, 42.08, 40.74, 38.08, 35.90, 34.63, 30.99, 28.45, 27.31, 26.63, 26.52, 23.01, 18.55, 17.67, 13.45, 6.93, 4.93; IR (neat, cm^{-1}) 2952, 2876, 1684, 1476, 1367, 1166, 1085, 1019, 725; HRMS m/z [M+Na] calcd 473.3785 for $C_{28}H_{54}O_2SiNa^+$, found 473.3873.

To a solution of *tert*-butyl ketone (40 mg, 0.0887 mmol) in THF (3 mL), a solution of tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 0.89 mL, 0.89 mmol) was added. After being stirred for 4 h at room temperature, the reaction mixture was concentrated. Purification of the residue by flash column chromatography (20% EtOAc/hexanes) provided 29.6 mg (0.088 mmol, 99% yield) of the corresponding alcohol. A solution of this alcohol (29.6 mg) in CH_2Cl_2 (3 mL) was added to pyridinium dichromate (PDC, 66.7 mg, 0.177 mmol, 2 equiv) and Celite (70 mg) at room temperature under argon atmosphere. After being stirred overnight, the reaction mixture was diluted with EtOAc and filtered through a silica gel plug. The filtrate was concentrated in vacuo and then purified by column chromatography (20% EtOAc/petroleum ether) to give 27.6 mg of the diketone **22** (0.0825 mmol, 94% yield) as colorless oil: $[\alpha]_D^{24}$ +8.7 (c 1.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.42 (dd, J = 11.4, 7.4 Hz, 1H), 2.29–2.16 (m, 2H), 2.09 (dm, J = 13.2 Hz, 1H), 2.03–1.81 (m, 3H), 1.76–1.39 (m, 8H), 1.37–1.14 (m, 2H), 1.23 (s, 3H), 1.21 (s, 9H), 1.20 (s, 3H), 0.94 (d, J = 6.4 Hz, 3H), 0.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 218.68, 212.04, 61.93, 56.27, 49.84, 49.34, 45.70, 40.93, 38.91, 38.12, 36.01, 30.96, 28.36, 27.46, 26.59, 26.55, 24.02, 19.05, 18.66, 12.41; IR (neat, cm^{-1}) 2957, 2873, 1715, 1682, 1477, 1385, 1366, 1220, 1044, 982; HRMS m/z [M+Na] calcd 357.2764 for $C_{22}H_{38}O_2Na^+$, found 357.2750.

4.16. 24-$(CH_3)_2$-25(O)TB analog (+)-4b

Phosphine oxide (−)-**11** and enantiomerically pure C,D-ring diketone **22** were separately azeotropically dried four times with benzene and held under vacuum for 60 h immediately prior to use. To a solution of phosphine oxide (−)-**11** (62.0 mg, 0.106 mmol) in THF (2 mL), a 1.6 M solution of *n*-BuLi in hexanes (66 μL, 0.106 mmol) was added dropwise at −78 °C under argon atmosphere. The resulting deep reddish-orange solution was allowed to stir for 20 min, at which time a precooled (−78 °C) solution of enantiomerically pure C,D-ring diketone (+)-**22** (15.8 mg, 0.0472 mmol) in THF (2 mL) was transferred dropwise via cannula. The deep reddish-orange solution was stirred in the dark for 5 h, during which time the color was faded. On observation of a light yellow color, the reaction mixture was quenched at −78 °C with 3 mL of buffer water (pH 7). The mixture was allowed to come up to rt, extracted with EtOAc (4× 10 mL), dried over Na_2SO_4, filtered,

concentrated, and purified by silica gel column chromatography (5% EtOAc/petroleum ether) to give 17 mg of the coupled product (0.0243 mmol, 52% yield).

This coupled product was dissolved in THF (2 mL) and treated with a solution of tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 0.24 mL, 0.24 mmol). After being stirred overnight at room temperature, the reaction mixture was concentrated. Purification of the residue by flash column chromatography (100% EtOAc) provided 9.9 mg of 24-$(CH_3)_2$-25(*O*)TB **4b** (0.021 mmol, 86% yield) as a white solid: mp (°C) 109–112; $[\alpha]_D^{24}$ +40.7 (*c* 0.48, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.37 (d, J = 11.2 Hz, 1H), 6.01 (d, J = 11.2 Hz, 1H), 6.07 (d, J = 16.0 Hz, 1H), 5.32 (m, 1H), 5.00 (m, 1H), 4.43 (m, 1H), 4.24–4.22 (m, 1H), 2.81 (dd, J = 12.0, 4.0 Hz, 1H), 2.59 (dd, J = 13.2, 3.2 Hz, 1H), 2.31 (dd, J = 13.4, 6.6 Hz, 1H), 2.05–1.81 (m, 5H), 1.72–1.43 (m, 10H), 1.32–1.19 (m, 4H), 1.23 (s, 3H), 1.22 (s, 9H), 1.21 (s, 3H), 0.91 (d, J = 6.0 Hz, 3H), 0.52 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 218.85, 147.60, 143.18, 132.81, 124.99, 116.99, 111.77, 70.82, 66.85, 56.30, 56.17, 49.45, 45.86, 45.25, 42.83, 40.41, 38.10, 36.64, 31.06, 29.06, 28.43, 27.58, 26.66, 26.51, 23.57, 22.25, 20.70, 18.78, 11.95; IR (neat, cm^{-1}) 3350, 2949, 2872, 1682, 1475, 1054, 755; HRMS *m*/*z* [M+Na] calcd 493.3652 for $C_{31}H_{50}O_3Na^+$, found 493.3675; UV (MeOH) λ_{max} = 265 nm (ε = 14,822).

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