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

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Abstract—Four new side-chain amide (**2** and **3**) and hydroxamate (**4** and **5**) analogs of the hormone calcitriol (**1**) have been prepared. Even though lacking the 25-OH group characteristic of natural calcitriol (**1**), analogs **2**–**4** are as antiproliferative in vitro as calcitriol (**1**) but are 20–40 times less calciuric in vivo than calcitriol (**1**).

1. Introduction

The natural hormone 1α,25-dihydroxyvitamin D_3 (**1**, calcitriol) regulates diverse biochemical functions, such as immunomodulation, the control of hormonal systems, inhibition of cell growth, and induction of cell differentiation.[3–5] Maintaining good health requires low levels of calcitriol, but using this hormone chemotherapeutically at supraphysiological levels is not feasible because of life-threatening hypercalcemia.[1,2] Therefore, medicinal organic chemists have developed a series of structurally modified versions of calcitriol that are at least as antiproliferative as calcitriol but substantially less calciuric than calcitriol.[4]

The vitamin D_3 side chain appears to be conformationally flexible with over 1083 low-energy conformers plausible.[6] Knowledge about side-chain flexibility gives the designer a point of attack, and recently we reported a series of highly growth-inhibitory, low-calcemic, side-chain ketone analogs of calcitriol.[7] These ketone analogs, as well as some sulfone[8] and sulfoximine[9] analogs, represent a group of analogs that lack the traditional terminal hydroxyl group and still maintain biological activity. Here, we report the synthesis and preliminary biological evaluation of a new series **2**–**5** of 16,17-saturated side-chain amide and hydroxamate analogs of calcitriol (**1**), in which the natural 25-OH is absent.

2. Results

2.1. Chemistry

Syntheses of analogs **2**–**5** are outlined in Schemes 1 and 2. In Scheme 1, selective N-alkylation allowed conversion of primary iodide **6**[10] into side-chain amides **7** and **8**, and into side-chain hydroxamate **9**. Horner–Wadsworth–Emmons (HWE) coupling of the A-ring phosphine oxide **13**[11] occurred as expected exclusively at the C-8 ketone carbonyl group. In Scheme 2, metal-promoted Michael addition of iodide **14**[12] to methyl acrylate produced 25-carbonyl adduct **15**. HWE coupling of NH-hydroxamate **17** with A-ring phosphine oxide **13** proceeded in modest yield.

2.2. Biology

Our standard in vitro murine keratinocyte proliferation assay[13,14] shows that amide analogs **2** and **3** and hydroxamate analog **4**, even though lacking the natural 25-OH group, are as cell growth-inhibitory as calcitriol (Fig. 1). Noteworthy are analogs **2**–**4** which show better activity than calcitriol, specifically at low-nanomolar concentrations. However, reversing the hydroxamate

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Keywords: Vitamin D; Calcitriol; Antiproliferative.

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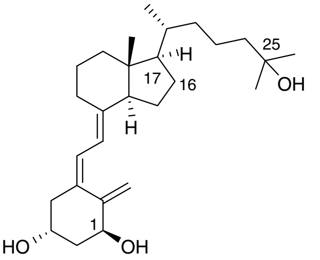
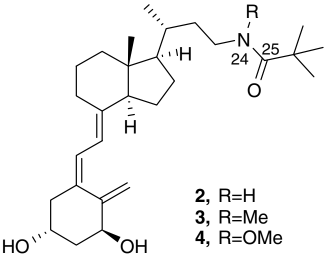
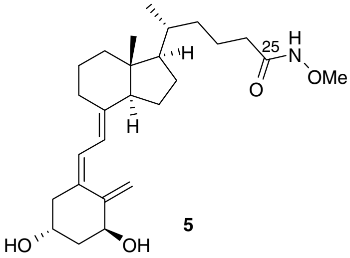


1(1α,25-dihydroxyvitamin D_3, calcitriol)

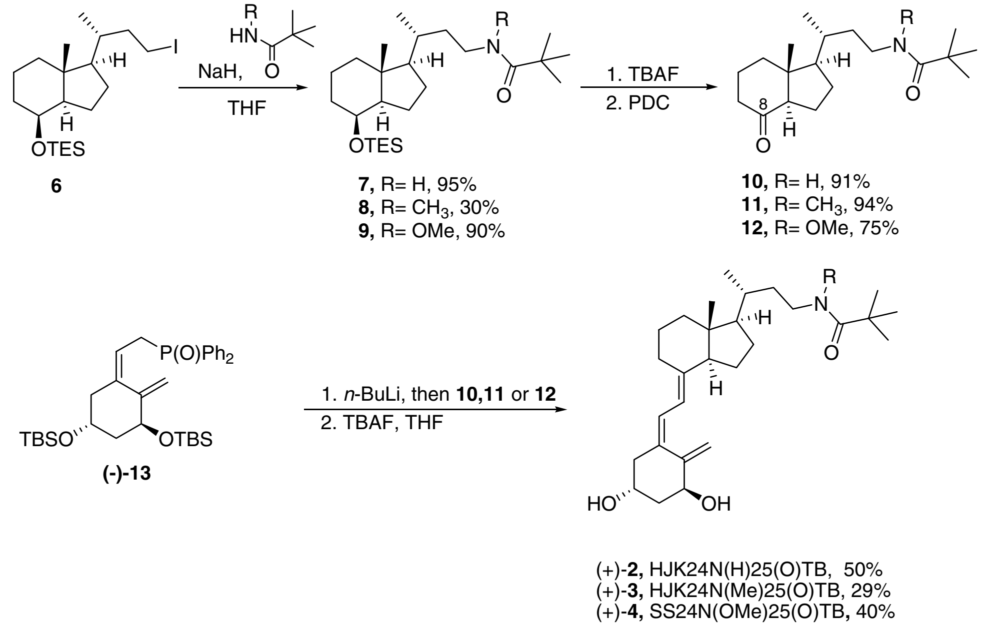


2, R=H
3, R=Me
4, R=OMe

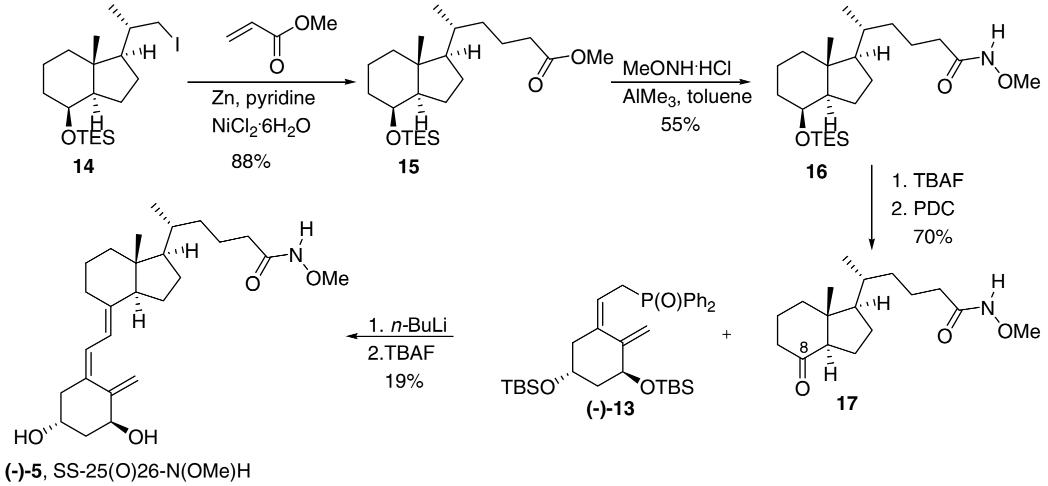


5

moiety, as in analog **5**, is not favorable for biological activity; analog **5** is less antiproliferative than calcitriol (Fig. 1). Our standard in vivo rat urine assay[13,14] shows 20–40 times higher doses (10–20 μg/kg) of the amide and hydroxamate analogs **2**–**4** are required to cause the same



Scheme 1.



Scheme 2.

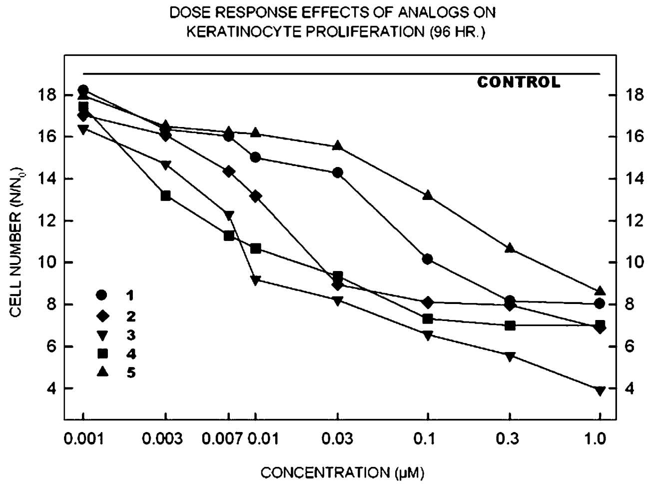


Figure 1.

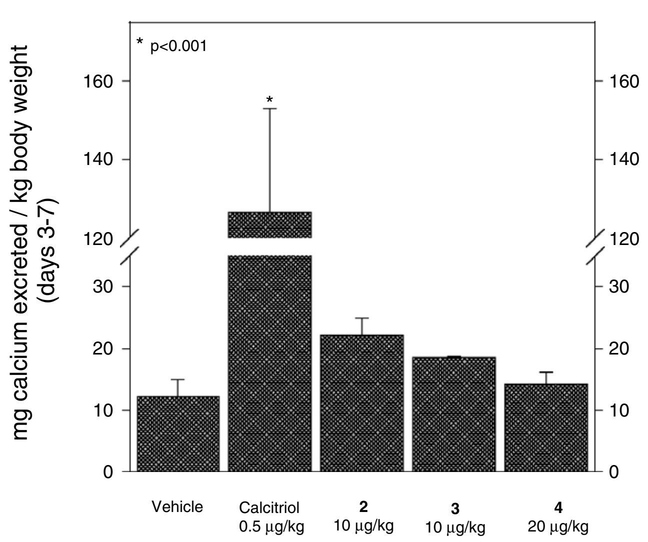


Figure 2.

level of urine calcium excretion as the natural hormone (0.5 µg/kg). The calciuric activities of the three analogs are similar to one another, with the least calciuric being the N–(OMe) analog **4**, as shown in Figure 2. Also, the analogs **2**–**4** do not compromise the weight gain of the rats even at doses that are more than 40 times higher than calcitriol (data not shown).

3. Conclusion

The new amide and hydroxamate analogs **2**–**4** show that terminating the C,D-ring side chain with a *tert*-butyl group instead of the natural tertiary 25-OH group and introducing a nitrogen atom at the 24 position in the form of a –N(R)C(O)– group produce analogs that have a favorable therapeutic window between desirably high antiproliferative activity and desirably low-calciuric activity. This desirable biological profile encourages further evaluation of amide and hydroxamate analogs **2**–**4** as potential chemotherapeutic agents for various human illnesses.

4. Experimental

All air and moisture sensitive reactions were carried out in flame-dried glassware under an inert atmosphere of argon. All reactive liquid reagents were transferred by syringe or cannula and were added into the flask through a rubber septum. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl immediately prior to use. All other solvents and reagents were used as received unless otherwise stated. *n*-BuLi was obtained from commercial sources and was titrated with *N*-pivaloyl-*O*-toluidine prior to use.

The ^{1}H spectra were obtained on a Varian XL 400 spectrometer at 400 MHz. The ^{13}C spectra were obtained on a Varian XL 100 spectrometer at 100 MHz. All NMR spectra were obtained as a solution in $CDCl_3$ with TMS as the internal standard unless otherwise stated. Chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS (0 ppm). Multiplicities of signals in the ^{1}H NMR spectra are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), dt (doublet of triplet), etc.

Infrared spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer as liquid films and thin layer with NaCl cells.

Optical rotations were recorded on JASCO, P-1100 model polarimeter (Japan Spectroscopic Co., Ltd) with sodium D line at the temperatures as indicated for the specific compounds.

Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates (Merck Kieselgel, 60, 0.25 mm thickness) with F_{254} indicator. Compounds were visualized under UV lamp and/or by developing with iodine, vanillin, *p*-anisaldehyde or $KMnO_4$. Flash chromatography was performed on 230–400 mesh silica gel (E. M. Science) with technical and/or HPLC grade solvents. Medium pressure liquid chromatography (MPLC) was performed with a FMI pump and prepacked silica gel column (Merck, Labor Columns, LiChroprep Si 60, 40–63 µm). High pressure liquid chromatography (HPLC) was performed on a Rainin HPLX system equipped with two 25 mL pump heads and a Rainin Dynamax UV-C dual-beam variable wavelength detector set at 254 or 260 nm using a Phenomenex, Luna 5 µ C18 semipreparative (250 × 10 mm) column or Chiralcel OJ semipreparative (250 × 10 mm) column.

HRMS were obtained at the mass spectrometry facility at the Ohio State University on a Micromass QTOF Electrospray mass spectrometer, or at The Johns Hopkins University on a VG70S double focusing magnetic sector FAB mass spectrometer (VG Analytical, Manchester, UK, now Micromass/Waters).

4.1. Synthesis of amide 2

4.1.1. Preparation of amide (+)-7. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with 2,2-dimethylpropionamide (51 mg, 0.50 mmol) and dissolved in 5 mL of freshly distilled THF. To this solution, sodium hydride (12 mg, 0.50 mmol) was added and was allowed to stir for 30 min before iodide **(+)-6**[12] (45 mg, 0.10 mmol) was cannulated into the flask as a solution in THF (2 mL). The mixture was gradually heated to reflux for 20 h. Thin-layer chromatography (TLC) showed the complete consumption of starting material. The reaction was quenched by addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL) and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography with EtOAc–hexanes (1/4), to afford 40 mg (95%) of amide **7** as a colorless oil. Data for **7**: $[\alpha]_D^{25}$ +46.7 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.53 (br s, 1H), 4.02 (m, 1H), 3.31 (m, 1H), 3.14 (m, 1H), 1.93 (dm, J_d = 12.4 Hz, 1H), 1.74–1.86 (m, 2H), 1.66 (m, 1H), 1.51–1.62 (m, 2H), 1.29–1.45 (m, 4H), 1.16–1.25 (m, 3H), 1.18 (s, 9H), 0.99–1.13 (m, 2H), 0.94 (t, *J* = 8.0 Hz, 9H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.89 (s, 3H), 0.54 (q, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 69.3, 56.5, 53.0, 42.1, 40.7, 38.5, 37.0, 35.5, 34.5, 33.4, 27.6, 27.4, 22.9, 18.7, 17.6, 13.4, 6.9, 4.9; IR (neat, cm^{-1}) 3342, 2952, 2875, 1637, 1537, 1458, 1373, 1230, 1165, 1083, 1025, 725; HRMS: calcd for $C_{25}H_{49}NO_2SiNa^+$ [M+Na$^+$]: 446.3425, found: 446.3439.

4.1.2. Preparation of ketone (+)-10. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with the TES protected alcohol **7** (38 mg, 0.090 mmol) dissolved in 3 mL of freshly distilled THF. To this solution, 0.35 mL of tetrabutylammonium fluoride (TBAF) (0.350 mmol, 1.0 M solution in THF) was added dropwise over several minutes and allowed to stir overnight at room temperature. TLC showed the complete consumption of starting material. The reaction was quenched by the addition of 5 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL), and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product. The residue was subjected to column chromatography with EtOAc–hexanes (1/1) as eluent to give the desired alcohol as a colorless oil.

The alcohol was charged into an argon purged 10 mL recovery flask equipped with a magnetic stir bar, a septum and dissolved in 4 mL of freshly distilled CH_2Cl_2. To this solution were added PDC (35 mg, 0.096 mmol) and 26 mg of oven-dried Celite in one portion at room temperature. The resulting mixture was allowed to stir at room temperature for about 12 h. TLC showed the complete consumption of starting material. The mixture was directly purified by flash chromatography with EtOAc–hexanes (34%) to afford 25 mg (91%) of ketone **10** as an oil. Data for **10**: $[\alpha]_D^{25}$ +12.7 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.64 (br s, 1H), 3.29 (m, 1H), 3.13 (m, 1H), 2.41 (dd, *J* = 11.6, 7.2 Hz, 1H), 2.14–2.27 (m, 2H), 2.07 (ddd, *J* = 13.2, 4.0, 2.4 Hz, 1H), 1.79–2.02 (m, 3H), 1.69 (m, 1H), 1.38–1.60 (m, 5H), 1.21–1.31 (m, 2H), 1.15 (s, 9H), 0.97 (d, *J* = 6.0 Hz, 3H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.8, 178.2, 61.8, 56.2, 49.8, 40.8, 38.8, 38.5, 36.8, 35.5, 33.6, 27.5, 23.9, 18.9, 18.7, 12.3; IR (neat, cm^{-1}) 3352, 2958, 2873, 1712, 1640, 1532, 1479, 1378, 1308, 1230, 1214, 1056; HRMS: calcd for $C_{19}H_{33}NO_2Na^+$ [M+Na$^+$]: 330.2403, found: 330.2415.

4.1.3. Preparation of HBJ-24N(H)25(O)TB (+)-2. Enantiomerically pure phosphine oxide **(−)-13** and CD-ring ketone **(+)-10** were separately azeotropically dried with anhydrous benzene (4× 5 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 **(−)-13** (47 mg, 0.081 mmol) and dissolved in 1.5 mL of freshly distilled THF to give a ca. 0.07 M solution. The flask was cooled to −78 °C in an isopropanol/dry ice bath. To this solution *n*-BuLi (50 μL, 0.081 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes during which time a deep red color 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 CD-ring ketone **(+)-10** (8.0 mg, 0.026 mmol) dissolved in 0.75 mL of freshly distilled THF and cooled to −78 °C in an isopropanol/dry ice bath. The solution of CD-ring ketone 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 ca. 5 h, during which time it was checked visually. Upon observation of a light yellow color, the reaction was quenched at −78 °C by the addition of 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL) and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography with EtOAc–hexanes (1/4) to afford 8.0 mg (53.0%) of product. The O-silylated analog was transferred into a flame-dried 5 ml flask, equipped with a stir bar, and purged with argon. It was then dissolved in 3 mL THF and cooled to −78 °C, and TBAF (0.13 mL, 0.13 mmol) was added dropwise. The mixture was gradually warmed to room temperature and left to stir overnight. The next day TLC showed complete consumption of starting material. The reaction was quenched by the addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography (100% ethyl acetate) to afford 5.0 mg of analog **2** as an oil (50% yield for two steps). Data for **2**: $[\alpha]_D^{25}$ +20 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, *J* = 11.2 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 5.54 (br s, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.32 (m, 1H), 3.17 (m, 1H), 2.82 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.60 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.31 (dd, *J* = 13.2, 6.4 Hz, 1H), 1.89–2.04 (m, 5H), 1.42–1.71 (m, 7H), 1.14–1.35 (m, 6H), 1.19 (s, 9H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 147.6, 143.0, 132.9, 124.9, 117.1, 111.8, 70.8, 66.8, 56.3, 56.3, 45.9, 45.3, 42.8, 40.4, 38.6, 37.1, 35.7, 34.3, 29.0, 27.8, 27.6, 23.5, 22.2, 18.9, 11.9; IR (neat, cm^{-1}) 3346, 2949, 2872, 1635, 1539, 1455, 1437, 1349, 1214, 1057, 754; HRMS: calcd for $C_{28}H_{45}NO_3$ Na^+ [M+Na^+]: 466.3292, found: 466.3312, λ_{max} 265 nm (ε 10,780).

4.2. Synthesis of amide 3

4.2.1. Preparation of ketone (+)-11. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with *N*-methyltrimethylamide (65 mg, 0.56 mmol) and dissolved in 5.6 mL of freshly distilled THF. To this solution sodium hydride (14 mg, 0.56 mmol) was added and allowed to stir for 30 min before iodide (+)-**6** (50 mg, 0.11 mmol) was cannulated into the flask as a solution in THF (2 mL). The mixture was gradually heated to reflux for 20 h. TLC showed the complete consumption of starting material. The reaction was quenched by the addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography with EtOAc–hexanes (1/6) to afford 13 mg (30%) of the amide **8** as a colorless oil.

A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with amide **8** (38 mg, 0.090 mmol) and dissolved in 3 mL of freshly distilled THF. To this solution 0.35 mL of tetrabutylammonium fluoride (TBAF) (0.35 mmol, 1.0 M solution in THF) was added dropwise over several minutes and the contents in the flask were left to stir overnight at room temperature. TLC showed the complete consumption of starting material. The reaction was quenched by the addition of 5 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL), and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product. The residue was purified by column chromatography with EtOAc–hexanes (1/1) to give the desired alcohol as a colorless oil. The alcohol was charged into an argon purged 10 mL recovery flask equipped with a magnetic stir bar, a septum and was dissolved in 3 mL of freshly distilled CH_2Cl_2. To this solution were added PDC (37 mg, 0.10 mmol) and 37 mg of oven-dried Celite in one portion at room temperature. The resulting mixture was allowed to stir at room temperature for about 12 h. TLC showed the complete consumption of starting material. The mixture was directly purified by flash chromatography with EtOAc–hexanes (1/2) affording 9.0 mg of ketone **11** as an oil (94%). Data for **11**: $[\alpha]_D^{25}$ +8.1 (*c* 0.50, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.26–3.43 (m, 2H), 3.01 (s, 3H), 2.44 (dd, *J* = 11.6, 7.6 Hz, 1H), 2.17–2.30 (m, 2H), 2.10 (ddd, *J* = 12.8, 4.4, 2.4 Hz, 1H), 1.85–2.04 (m, 3H), 1.26–1.78 (m, 8H), 1.26 (s, 9H), 1.02 (d, *J* = 6.0 Hz, 3H), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.9, 177.1, 61.9, 56.4, 49.9, 47.9, 40.9, 38.9, 38.7, 36.3, 33.9, 32.9, 28.3, 27.6, 24.0, 19.0, 18.9, 12.4; IR (neat, cm^{-1}) 2957, 2874, 1713, 1626, 1480, 1404, 1378, 1367, 1307, 1116, 1080.

4.2.2. Preparation of HBJ-24N(Me)25(O)TB (+)-3. Enantiomerically pure phosphine oxide (−)-**13** and CD-ring ketone (+)-**11** were separately azeotropically dried with anhydrous benzene (4× 5 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 (−)-**13** (35 mg, 0.060 mmol) and dissolved in 1 mL of freshly distilled THF to give a ca. 0.06 M solution. The flask was cooled to −78 °C in an isopropanol/dry ice bath. To this solution *n*-BuLi (38 μL, 0.060 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes, during which time a deep red color 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 CD-ring ketone (+)-**11** (9.0 mg, 0.028 mmol) dissolved in 1 mL of freshly distilled THF and cooled to −78 °C in an isopropanol/dry ice bath. The solution of CD-ring ketone 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 ca. 4 h during which time it was checked visually. Upon observation of a light yellow color, the reaction was quenched at −78 °C by the addition of 5 mL of pH 7 buffer and allowed to warm 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 (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography with EtOAc–hexanes (1/6) to afford 6.0 mg of product (31%). The O-silylated analog was transferred into a flame-dried 5 ml flask, equipped with a stir bar, and purged with argon. Then, the product was dissolved in 2 mL THF and cooled to −78 °C, and TBAF (0.09 mL, 0.09 mmol) was added dropwise. The mixture was gradually warmed up to room temperature and left to stir overnight. The next day TLC showed complete consumption of starting material. The reaction was quenched by the addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to afford the crude product which was purified by flash chromatography with EtOAc (100%) to afford 4.0 mg of analog **3** as an oil (29% yield for two steps). Data for **3**: $[\alpha]_D^{25}$ +62 (*c* 0.10, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, *J* = 11.2 Hz, 1H), 6.01 (d, *J* = 11.2 Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.43 (m, 1H), 4.23 (m, 1H), 3.29–3.42 (m, 2H), 3.01 (s, 3H), 2.82 (dd, *J* = 12.0, 4.0 Hz, 1H), 2.60 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.31 (dd, *J* = 13.2, 6.4 Hz, 1H), 1.89–2.01 (m, 5H), 1.22–1.71 (m, 11H), 1.27 (s, 9H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 147.6, 143.0, 133.0, 124.9, 117.1, 111.8, 70.8, 66.8, 56.30, 56.27, 48.0, 45.9, 45.2, 42.8, 40.4, 38.7, 36.3, 34.5, 33.1, 29.0, 28.3, 27.7, 23.5, 22.2, 19.1, 11.9; IR (neat, cm^{-1}) 3388, 2946, 2872, 1608, 1408, 1364, 1261, 1214, 1114, 1056, 753; HRMS: calcd for $C_{29}H_{48}NO_3$ $[M+H^+]$: 458.3556, found: 458.3634; λ_{max} 265 nm (ε 12,287).

4.3. Synthesis of hydroxamate 4

4.3.1. Preparation of ketone (+)-12. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with *tert*-butyl(*O*-methyl) hydroxamate (88 mg, 0.67 mmol) and dissolved in 5 mL of freshly distilled DMF, making a 1.34 M solution. To this solution, sodium hydride (17 mg, 0.74 mmol) was added and was allowed to stir for 30 min before iodide (+)-**6** (27 mg, 0.67 mmol) was cannulated into the flask as a solution in DMF (1.5 mL). The mixture was gradually heated to reflux for 24 h. TLC showed the complete consumption of starting material. The reaction was quenched by addition of 15 mL of distilled water at room temperature and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography with EtOAc–hexanes (1/4) to afford 22 mg (89%) of the hydroxamate **9** as a colorless oil.

A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged with hydroxamate **9** (22 mg, 0.049 mmol) and dissolved in 2 mL of freshly distilled THF. To this solution 0.10 mL of tetrabutylammonium fluoride (TBAF) (0.10 mmol, 1.0 M solution in THF) was added dropwise over several minutes and the contents of the flask were left to stir overnight at room temperature. TLC showed the complete consumption of starting material. The reaction was quenched by the addition of 5 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL), and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography with EtOAc–hexanes (1/10) to give 22 mg (82%) of alcohol as an oil.

The alcohol was charged into an argon purged 10 mL recovery flask equipped with a magnetic stir bar, a septum and dissolved in 3 mL of freshly distilled CH_2Cl_2. To this solution were added PDC (27 mg, 0.070 mmol) and 27 mg of oven-dried Celite in one portion at room temperature. The resulting mixture was allowed to stir at room temperature for about 12 h. TLC showed the complete consumption of starting material. The mixture was directly purified by flash chromatography with EtOAc–hexanes (1/10) to afford 12 mg (91%) of ketone **12** as an oil. Data for **12**: $[\alpha]_D^{25}$ +11.4 (*c* 4.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.27–4.24 (dd, 2H, *J* = 6 Hz, *J* = 7.6 Hz), 3.72 (s, 3H), 2.48–2.43 (dd, 1H, *J* = 7.6 Hz, *J* = 11.6 Hz), 2.31–2.17 (m, 2H), 2.10 (d, 1H, *J* = 12.8 Hz), 2.05–1.97 (m, 1H), 1.96–1.91 (m, 1H), 1.63–1.49 (m, 3H), 1.11 (s, 9H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.9, 162.3, 70.6, 61.9, 61.5, 56.8, 52.9, 49.9, 46.6, 40.9, 38.9, 36.3, 36.1, 32.6, 27.6, 27.5, 24.0, 19.0, 18.9, 12.4; IR (neat, cm^{-1}) 2962, 2873, 1717, 1634, 1462, 1378, 1362, 1300, 1162, 1056, 901; HRMS: calcd for $C_{20}H_{35}NO_2$ Na^+ [M+Na]: 360.2509, found: 360.2513.

4.3.2. Preparation of SS-24N(OMe)25(O)TB (+)-4. Enantiomerically pure phosphine oxide (−)-**13** and CD-ring ketone (+)-**12** were separately azeotropically dried with anhydrous benzene (4× 5 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 (−)-**13** (60 mg, 0.10 mmol) and dissolved in 1 mL of freshly distilled THF to give a ca. 0.1 M solution. The flask was cooled to −78 °C in an isopropanol/dry ice bath. To this solution *n*-BuLi (60 μL, 0.10 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes during which time a deep red color 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 CD-ring ketone (+)-**12** (12 mg, 0.030 mmol) dissolved in 1 mL of freshly distilled THF and cooled to −78 °C in an isopropanol/dry ice bath. The solution of CD-ring ketone 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 ca. 2 h during which time it was checked visually. Upon observation of a light yellow color, the reaction was quenched at −78 °C by the addition of 5 mL of pH 7 buffer and allowed to warm to room temperature. The mixture was then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL) and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography EtOAc–hexanes (1/10) to afford 20 mg of product (78%). The O-silylated analog was transferred into a flame-dried 5 ml flask, equipped with a stir bar, and purged with argon. The product was then dissolved in 2 mL THF and cooled to −78 °C, and TBAF (0.30 mL, 0.30 mmol) was added dropwise. The mixture was gradually warmed to room temperature and left to stir overnight. The next day TLC showed complete consumption of starting material. The reaction was quenched by the addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash column chromatography (50% ethyl acetate in hexanes) to afford 5.0 mg of analog **4** as an oil (40% yield for two steps). Data for **4**: $[\alpha]^{24}_{D}$ +58 (*c* 0.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, 1H, $J = 11.2$ Hz), 6.01 (d, 1H, $J = 11.2$ Hz), 5.33 (s, 1H), 5.00 (s, 1H), 4.44 (m, 1H), 4.27–4.2 (m, 3H), 3.72 (s, 3H), 2.82 (dd, 1H, $J = 4.0$, 12.4 Hz), 2.60 (dd, 1H, $J = 3.2$, 13.2 Hz), 2.31 (dd, 1H, $J = 6.4$, 13.2 Hz), 2.08–1.8 (m, 6H), 1.72–1.45 (m, 8H), 1.37–1.22 (m, 4H), 1.15 (s, 9H), 0.96 (d, 3H, $J = 6.4$ Hz), 0.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 147.6, 143.0, 133.0, 124.9, 117.1, 111.8, 70.8 (d, $J = 6.8$ Hz), 66.8, 61.5, 56.6, 56.3, 45.9, 45.2, 42.8, 40.4, 36.3, 36.2, 33.1, 29.7, 29.0, 28.3, 27.7, 27.5, 25.8, 23.5, 22.3, 19.0, 11.9; IR (neat, cm^{-1}) 3371.4, 2940.9, 2874.6, 1737.5, 1627.1, 1461.5, 1373.2, 1295.9, 1240.7, 1157.9, 1058.6, 893.0; HRMS: calcd for $C_{29}H_{47}NO_4\,Na^+$ [M+Na]: 496.3397, found: 496.3397; λ_{max} 265 nm (ε 15,954).

4.4. Synthesis of hydroxamate 5

4.4.1. Preparation of dicarbonyl (+)-16. In a flame-dried 25 mL two-necked round-bottomed flask equipped with a magnetic stir bar, a septum, a reflux condenser, and an Ar balloon were placed activated zinc (0.086 g, 1.3 mmol), methyl acrylate **14** (0.14 mL, 1.54 mmol), and $NiCl_2{\cdot}6H_2O$ (0.040 g, 0.17 mmol) in pyridine (3 mL). The mixture was heated at 65 °C for 1 h, during which time the color of the reaction mixture turned reddish brown. The reaction mixture was cooled to 0 °C and added a solution of pre-cooled (0 °C) 22-iodide (+)-**6** (0.050 g, 0.11 mmol). It was then warmed to room temperature and allowed to stir for 3 h. The mixture was then diluted with EtOAc (5 mL) and filtered through a pad of Celite. The filtrate was washed with 5% HCl (2× 5 mL) and extracted with EtOAc (3× 5 mL). The combined extracts were washed with water (1× 5 mL) and brine (1× 5 mL), dried over $NaSO_4$, and concentrated in vacuo. The crude product was purified via flash chromatography using EtOAc–hexanes (1/10) to afford 38 mg (88%) of ester **15**, as an oil. For the preparation of the 25(O)26N(OMe)H-CD-ring-8-OTES **16**,[4] the purified ester **15** was transferred into a 10 mL pear-shaped flask and placed under vacuum for 24 h. In a flame-dried 25 mL round-bottomed flask equipped with a magnetic stir bar, a septum and an argon balloon was placed well-dried methoxylamine hydrochloride (0.007 g, 0.08 mmol) and dissolved in 10 mL toluene to give a ca. 0.08 M solution. This mixture was cooled to 0 °C before trimethylaluminum (0.042 mL, 0.085 mmol) was added dropwise. This was allowed to stir at 0 °C for 5 min, before the reaction was allowed to warm to room temperature and left to stir for 1 h before ester **15** (0.030 g, 0.076 mmol) was added via a cannula as a 0.08 M solution in benzene. This was allowed to stir for 5 h. TLC showed the complete consumption of starting material. The reaction was quenched with water (1× 10 mL), neutralized with 1 M HCl, and extracted with ethyl acetate (3× 30 mL). The extracts were combined, dried over $NaSO_4$, filtered, and concentrated in vacuo. The crude product was purified via flash chromatography using EtOAc–hexanes (1/2) to afford 21 mg (71%) of ester **16** as a colorless oil. Data for **16**: $[\alpha]^{25}_{D}$ +34 (*c* 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 4.02–4.01 (d, 1H, $J = 2.4$ Hz), 3.76 (s, 3H), 1.95–1.91 (m, 1H), 1.86–1.04 (m, 18H), 0.96–0.89 (m, 15H), 0.57–0.51 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 69.5, 62.9, 56.6, 53.0, 41.9, 40.7, 35.0, 34.9, 34.3, 32.7, 26.92, 22.8, 17.6, 17.3, 12.8, 5.93, 4.5; IR (neat, cm^{-1}) 3177, 2944, 2875, 1657, 1455, 1368, 1229, 1166, 1078, 1015, 971; HRMS: calcd for $C_{23}H_{45}NO_3\,SiNa^+$ [M+Na]: 434.3061, found: 435.3051.

4.4.2. Preparation of ketone (+)-17. A flame-dried 10 mL recovery flask equipped with a magnetic stir bar, a septum along with an Ar balloon was charged TES protected alcohol **16** (0.021 g, 0.049 mmol) and dissolved in 3 mL of freshly distilled THF. Then the flask was cooled to −78 °C in an isopropanol/dry ice bath. To this solution 0.485 mL of TBAF (0.49 mmol, 1.0 M solution in THF) was added dropwise over several minutes and the contents of the flask were allowed to stir at −78 °C for an additional 30 min. The mixture was gradually warmed to room temperature and left to stir overnight. TLC showed the complete consumption of starting material. The reaction was quenched by addition of 5 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 25 mL). The combined extracts were washed with water (1× 25 mL), and brine (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography EtOAc–hexanes (7/10) to afford 10 mg (71%) of deprotected alcohol. The purified alcohol was charged into an argon purged 10 mL recovery flask equipped with a magnetic stir bar, a septum and dissolved in 2 mL of freshly distilled THF to give a ca. 0.02 M solution. To this solution were added PDC (0.026 g, 0.071 mmol) and 16 mg of oven-dried Celite in one portion at room temperature. The resulting mixture was allowed to stir at room temperature for about 12 h. TLC showed the complete consumption of starting material. The mixture was directly purified by column chromatography with EtOAc/Hexanes (1/1) to afford 9.0 mg (89%) of ketone (+)-**17** as an oil. Data for **17**: $[\alpha]_{\mathrm{D}}^{25}$ +10 (c 0.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 3.77 (s, 3H), 2.46–2.41 (m, 1H), 2.33–2.16 (m, 3H), 2.12–1.97 (m, 3H), 1.95–1.82 (m, 3H), 1.77–1.43 (m, 10H), 1.34–1.28 (m, 3H), 0.98–0.96 (d, 3H, $J = 8$Hz), 0.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (not enough material to obtain a clean spectrum); IR (neat, cm^{-1}) 3210, 2956, 1711, 1652, 1456, 1378, 1070; HRMS: calcd for $C_{18}H_{33}NO_3\,Na^+$ [M+Na]: 346.2353, found: 346.2364.

4.4.3. Preparation of SS-23-oxa-24N(Me)25(O)TB-(5). Enantiomerically pure phosphine oxide (−)-**13** and CD-ring ketone (+)-**17** were separately azeotropically dried with anhydrous benzene (4× 5 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 and a septum with an Ar balloon was charged with phosphine oxide (−)-**13** (59 mg, 0.10 mmol) and dissolved in 0.75 mL of freshly distilled THF to give a ca. 0.13 M solution. The flask was cooled to −78 °C in an isopropanol/dry ice bath. To this solution, *n*-BuLi (71 μL, 0.11 mmol, 1.60 M solution in hexanes) was added dropwise over several minutes during which time a deep red color 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 CD-ring ketone (+)-**17** (10 mg, 0.034 mmol) dissolved in 0.75 mL of freshly distilled THF and cooled to −78 °C in an isopropanol/dry ice bath. The solution of CD-ring ketone 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 ca. 2 h during which time it was checked visually. Upon observation of a light yellow color, the reaction was quenched at −78 °C by addition of 5 mL of pH 7 buffer and allowed to warm 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 (1× 25 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by column chromatography (50% ethyl acetate in hexanes) to afford 5.0 mg (24%) of product.

The O-silylated analog was transferred into a flame-dried 5 mL flask, equipped with a stir bar and purged with argon. The product was then dissolved in 1 mL THF and cooled to −78 °C, and TBAF (0.040 mL, 0.075 mmol) was added dropwise. The mixture was gradually warmed to room temperature and left to stir overnight. The next day TLC showed the complete consumption of starting material. The reaction was quenched by the addition of 15 mL of distilled water and then rinsed into a separatory funnel with ethyl acetate. The mixture was extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with water (1× 5 mL), and brine (1× 5 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give the crude product which was purified by flash chromatography (100% ethyl acetate) to afford 1.4 mg of analog (−)-**5** as an oil (11% yield for two steps). $[\alpha]_{\mathrm{D}}^{24}$ −4.6 (c 0.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 6.39–6.36 (d, 1H, $J = 11.2$ Hz), 6.02–6.00 (d, 1H, $J = 10.8$ Hz), 5.33–5.32 (m, 1H), 5.00 (s, 1H), 4.44–4.43 (m, 1H), 4.24–4.22 (m, 1H), 3.76 (s, 1H), 2.84–2.81 (m, 1H), 2.62–2.59 (m, 1H), 2.35–2.27 (m, 1H), 2.04–1.85 (m, 7H), 1.68–1.05 (m, 17H), 0.95–0.94 (d, 3H, $J = 6.0$ Hz), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ insufficient material for a carbon NMR; HRMS: calcd for $C_{26}H_{41}NO_4\,Na^+$ [M+Na]: 454.2927, found: 454.2913; UV (MeOH) $\lambda_{\max}$ 265 nm (ε 15,958).

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