Conceptually New Low-Calcemic Oxime Analogues of the Hormone 1α,25-Dihydroxyvitamin D_3: Synthesis and Biological Testing

Gary H. Posner,*,† Bethany A. Halford,† Sara Peleg,‡ Patrick Dolan,§ and Thomas W. Kensler§

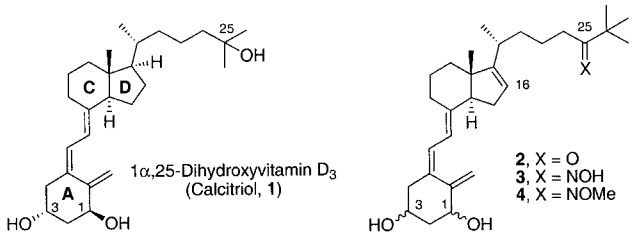
Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, Baltimore, Maryland 21218, Department of Medicinal Specialties, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030, and Department of Environmental Health Sciences, Division of Toxicological Sciences, School of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205

Received December 10, 2001

New chemical entities 16-ene-25-ketone **2b** and the corresponding oxime **3b** and oxime ether **4b**, analogues of natural calcitriol (**1**), were rationally designed and synthesized on a milligram scale. Chemical introduction of the oxime ether functionality in analogue **4b** was successful via direct oximation of an intact vitamin D conjugated triene system. Even though all three analogues are at least as antiproliferative in vitro as calcitriol (**1**) even at physiologically relevant low nanomolar concentrations, only side chain ketone **2b** is more transcriptionally potent than calcitriol (**1**). Although oxime O-methyl ether **4b** lacks the traditional side chain hydrogen bond-donating OH group of the natural hormone and lacks also the oxime−NOH group of analogue **3b**, surprisingly, oxime ether **4b** retains 20% of the transcriptional potency of natural calcitriol (**1**). In terms of in vivo toxicity (hypercalcemia), ketone **2b** is strongly calcemic in rats, whereas oxime **3b** and oxime ether **4b** are considerably less calcemic (i.e., safer) than calcitriol (**1**).

Introduction

Many analogues of the hormone 1α,25-dihydroxyvitamin D_3 (calcitriol, **1**) have been synthesized.[1] Several of these analogues currently are being evaluated as drug candidates for cancer chemoprevention[2] and chemotherapy[3] as well as for chemotherapy of other human diseases such as osteoporosis, psoriasis, and immune system disorders.[4,5] Successful drugs must have an acceptable therapeutic index, with a good separation between efficacy and toxicity (e.g., hypercalcemia) and desirable pharmacological properties (e.g., high metabolic stability).[4,5] Because the main sites of catabolism are located in the C,D-ring side chain of calcitriol (**1**),[1] analogues with catabolism-blocking side chain olefinic[6−8] and acetylenic[9] unsaturation, including side chain keto versions,[10,11] have been developed. No side chain oxime analogues, however, have been reported. We report here preparation of 25-keto analogue **2** and its oxime derivatives **3** and **4**. Our rationale for designing 16-ene 25-oxime analogues (**3**) and oxime O-methyl ether analogues (**4**) was based on three major considerations: (i) the 16-ene modification usually amplifies an analogue's biological potency;[12] (ii) the hydroxyl group of oximes **3** occupies the same region of space as the important 25-OH group of the natural hormone, and therefore oxime analogues **3** should bind well to the transporter D binding protein (DBP) and to the vitamin D receptor (VDR);[1] and (iii) some oximes and oxime O-ethers have desirable biological activities including antibiotic nocardicin,[13] an antibacterial pyrrolidine oxime O-methyl ether,[14] a muscarinic agonist oxime O-alkynyl ether,[15] a dehydroquinase inhibitor oxime,[16] anticonvulsant oxime ethers,[17] dioxygenase inhibitor sethoxydim,[18] mitochondrial complex I inhibitor oximes,[19] juvenile hormone biosynthesis inhibitor brevioxime,[20] an agricultural fungicide oxime O-methyl ether,[20] antihyperglycemic oximes ethers,[21] an antitumor oxime O-*tert*-butyl ether,[22] and a drug candidate oxime O-ethyl ether.[23] Some steroidal (but not vitamin D) oximes are natural products,[24] inhibitors of squalene-hopene cyclase,[25] superestrogens,[26] digitalis-like,[27] and cytochrome P450 inhibitors.[28,29]



Chemistry Structures

The oxime functionality can be installed, in principle, either before or after coupling of the A-ring with the C,D-ring. Before such coupling, installing the O-methyl oxime group into the C,D-ring side chain *tert*-butyl ketone **6** proceeded smoothly to give exclusively the (*E*)-oxime methyl ether **7** (stereochemical assignment based on ^{13}C nuclear magnetic resonance (NMR) spectroscopy),[30] due presumably to the strongly unfavorable steric congestion that would be present in the corresponding (*Z*)-oxime methyl ether.[30] Horner−Wadsworth−Emmons (HWE) coupling[6] of the corresponding enantiomerically pure C-8 ketone oxime methyl ether **8** with

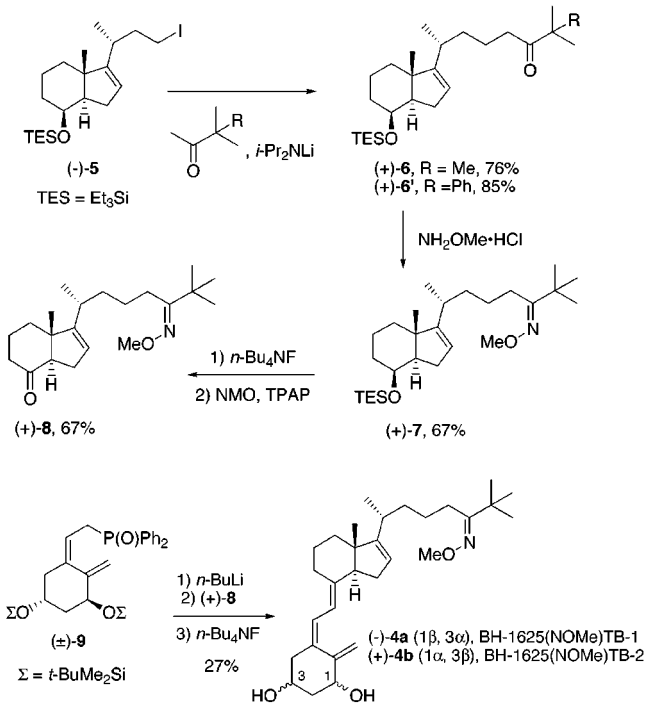
* To whom correspondence should be addressed. Tel.: 410-516-4670. Fax: 410-516-8420. E-mail: ghp@jhu.edu.
† Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University.
‡ Department of Medicinal Specialties, The University of Texas, M. D. Anderson Cancer Center.
§ Department of Environmental Health Sciences, Division of Toxicological Sciences, School of Medicine, The Johns Hopkins University.

10.1021/jm010560o CCC: $22.00
Published on Web 03/07/2002

Table 1. ^{1}H NMR (δ) and Optical Rotation (deg) Characteristics of Analogues

analogue	$C_{18}-CH_3$	C_6-H	C_7-H	$[\alpha]_D^{25}$
2a	0.68	6.37	6.09	+17.2
2b	0.67	6.39	6.09	−4.3
3a	0.69	6.38	6.10	+4.8
3b	0.68	6.39	6.09	−3.7
4a	0.69	6.38	6.10	+6.0
4b	0.69	6.38	6.09	−2.4

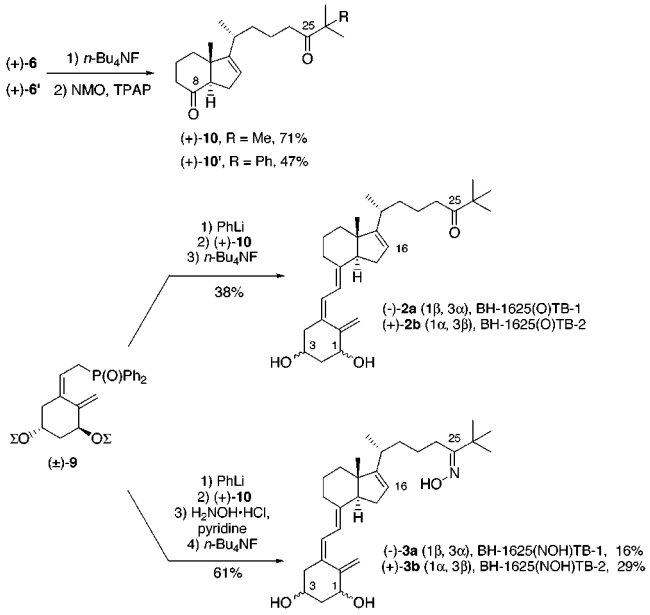
Scheme 1



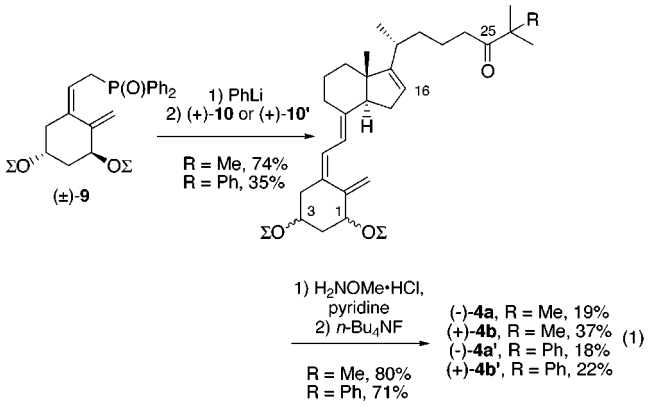
racemic A-ring phosphine oxide **9**[31] produced, after desilylation, calcitriol O-methyl oxime analogues **4** as two diastereomers, with enantiomerically pure diastereomer **4b** of natural A-ring stereochemistry predominating. As we have done generally before,[8,31] tentative assignment of the A-ring stereochemistry in analogues **4** was achieved based on characteristic ^{1}H NMR peaks and optical rotations (Table 1). The success of this convergent approach allows considerable diversity in preparation of a library of oxime O-ethers from the same A-ring component **9** and diverse 8-keto 25-oxime ethers **8**.

It was not possible, however, to use this approach to prepare the free oxime **3**. *tert*-Butyl ketone **6** did not react with hydroxylamine O-*tert*-butyldimethylsilyl ether, and oxidation of a C-8 alcohol side chain oxime did not produce much of the desired C-8 ketone 25-oxime. Therefore, installing the oxime functionality was postponed until after coupling of the A-ring and C,D-ring fragments. Thus, C-8,C-25-diketone **10** (Scheme 2) was chemospecifically mono-olefinated[11] at C-8 (due to steric hindrance at C-25) producing, after desilylation, mainly 25-keto analogue **2b** with natural A-ring stereochemistry. Likewise, HWE coupling of enantiomerically pure diketone **10** with racemic A-ring phosphine oxide **9** followed by direct reaction with hydroxylamine hydrochloride and then desilylation gave desired oxime analogues **3**, with natural A-ring diastereomer **3b** predominating. Importantly, the oxime installation step

Scheme 2



using hydroxylamine hydrochloride in pyridine solvent proceeded without destroying the acid-sensitive conjugated triene unit of this seco-steroid. This successful direct oximation of the 25-keto group of an intact vitamin D molecule suggests that such a terminal oximation step using diverse O-substituted hydroxylamines will be useful generally in producing a library of new and diverse 25-oxime ether analogues. As first proof of this principle, direct oximation of the 25-keto group of an intact calcitriol (**1**) analogue proceeded in high yield using hydroxylamine O-methyl ether to form analogue oxime O-methyl ethers **4a** and **4b**; similarly prepared were side chain phenyl oxime O-methyl ethers **4a′** and **4b′** (eq 1).



Analogues **2**−**4** exhibited the A-ring conformational equilibrium commonly observed in vitamin D compounds (eq 2).[12] The ratio of these two chair conformers was determined using the ^{1}H NMR vicinal coupling constant between H(3α)−H(4β) for analogues **2a**, **3a**, and **4a** and between H(3β)−H(4α) for analogues **2b**, **3b**, and **4b** (Table 2). This widely accepted method[32,33] uses reference data reported for the coupling constants

Table 2. ^{1}H NMR (δ), J_{H-H} Coupling Constants (Hz), and Ratio of A-Ring Chair Conformers[a]

analogue	C_3-H (m)	C_4-H_α (dd)	C_4-H_β (dd)	α:β
2a	4.22	2.30 (13.2, 7.6)	2.62 (13.2, 4.0)	42:58
2b	4.24	2.60 (13.0, 3.2)	2.31 (13.0, 6.0)	39:61
3a	4.22	2.28 (13.2, 6.0)	2.62 (13.2, 3.4)	61:39
3b	4.24	2.61 (13.6, 3.4)	2.32 (13.6, 6.4)	44:56
4a	4.22	2.30 (13.2, 8.0)	2.62 (13.2, 3.8)	37:63
4b	4.24	2.60 (13.4, 3.6)	2.32 (13.4, 6.0)	39:61

[a] Spectra were recorded at 400 MHz in $CDCl_3$.

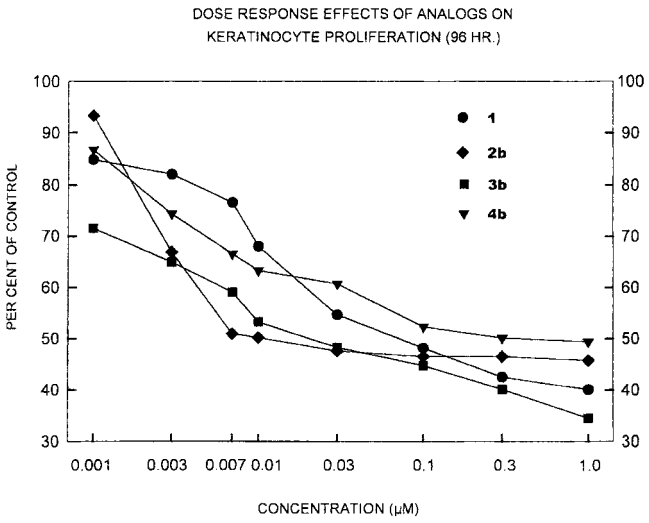
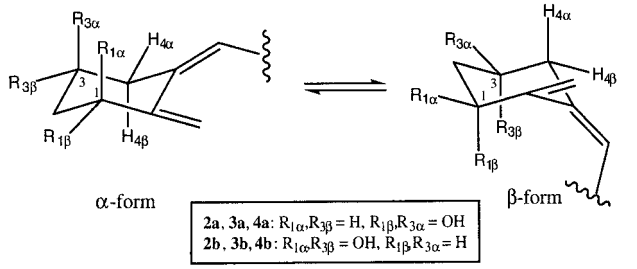


Figure 1.

of cyclohexanol protons ($J_{ax,ax}$ = 11.1 Hz, $J_{eq,eq}$ = 2.7 Hz).[34]



Biology

Our standard protocol for measuring in vitro antiproliferative activity in murine keratinocytes[31] showed that only three of the new analogue diastereomers with natural 1,3-diol stereochemistry (i.e., **2b**, **3b**, and **4b**) are antiproliferative, as seen also in other recent analogues.[8] Keto analogue **2b**, oxime **3b**, and oxime O-methyl ether **4b** are all at least as antiproliferative as calcitriol (**1**), even at low nanomolar concentrations (Figure 1). Terminal phenyl oxime O-methyl ether **4b′**, however, is not antiproliferative even at 300 nM concentration (data not shown).

VDR-mediated in vitro transcriptional activities of these new antiproliferative analogues were tested in rat osteosarcoma ROS 17/2.8 cells, using a standard protocol.[35] Ketone **2b** was found to be four times more potent (ED_{50} = 0.1 nM) than calcitriol (**1**, ED_{50} = 0.4 nM), whereas both oxime **3b** (ED_{50} = 10 nM) and oxime ether **4b** (ED_{50} = 2.0 nM) were less potent than calcitriol (**1**). The high transcriptional potency of ketone **2b** is especially noteworthy because it lacks the natural 25-OH group that for many years was considered essential for strong binding to the VDR and for high genomic activity.[1,11,36,37] Indeed, competitive binding affinities[38] for these analogues (relative to 100% binding of the natural hormone) are as follows: **2b**, 35%; **3b**, 3%; **4b**, <3%. Thus, even though ketone **2b** binds only moderately to the VDR, it is at least as antiproliferative as calcitriol (**1**), providing yet another example of an analogue in which there is no direct relationship between its VDR binding affinity and its transcriptional activity.[36,37] Furthermore, although oxime ether **4b** lacks a side chain OH group and has less than 3% of the VDR binding affinity of calcitriol (**1**), surprisingly, **4b** does have considerable transcriptional potency, 20% that of calcitriol (**1**).

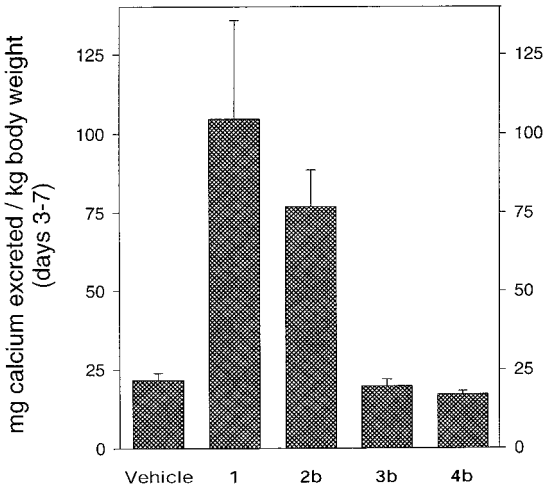


Figure 2. Effects of vitamin D_3 analogues on urinary calcium excretion in rats. Animals were treated with 0.5 μg/kg body weight of test compound po for 7 consecutive days, and urinary excretion of calcium was measured during days 3−7. Values are mean ± SE from three animals in each group.

As a measure of their safety in animals, each analogue **2b**, **3b**, and **4b** was administered orally to rats daily for 1 week at a similar dose (0.5 μg/kg body weight) to calcitriol (**1**).[38] Whereas keto analogue **2b** did significantly elevate levels of calcium in the blood of rats (i.e., hypercalemia), oxime **3b** and oxime ether **4b** did not cause elevation in the blood calcium levels (Figure 2).

In conclusion, new chemical entities **3b** and **4b** are the first members of a new family of oxime analogues of calcitriol (**1**) that may be used as sensitive molecular probes of the delicate relationship between chemical structure and biological activity.[39] Oxime O-methyl ether **4b** is also an important example, like our recent sulfone analogues,[38,40] showing that a side chain OH group is not necessary for a calcitriol (**1**) analogue to produce powerful biological responses. On the basis of the data presented here, oxime **3b** and oxime ether **4b** deserve more biological evaluation especially in vivo to establish their practical therapeutic and prophylactic potential in various human diseases.

Experimental Section

General. Unless otherwise noted, reactions were performed in oven-dried glassware under an atmosphere of ultrahigh-purity argon. Diethyl ether (Et_2O) and tetrahydofuran (THF) were distilled from sodium benzophenone ketyl immediately prior to use. Methylene chloride (CH_2Cl_2) and triethylamine (Et_3N) were distilled from calcium hydride prior to use. Organolithiums were titrated prior to use following known methods. All chemicals were purchased from commercial suppliers and used without further purification unless otherswise noted. Column chromatography was performed using short path silica gel (particle size <230 mesh.) High-performance liquid chromatography (HPLC) was carried out using a Rainin HPLX system equipped with two 25 mL/min preparative pump heads using Rainin Dynamax 10 mm × 250 mm (semipreparative) columns packed with 60 Å silica gel (8 μm pore size) as C-18-bonded silica and a Rainin Dynamax UV-C dual-beam variable-wavelength detector set at 265 nm. Yields are reported for pure products (>95% based on their chromatographic and spectroscopic homogeneity) and are unoptimized. Optical rotations were measured at the sodium line using a Perkin-Elmer 141 Polarimeter and are the average of seven values. NMR spectra were obtained on a Varian XL-400 spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ) and are referenced to $CDCl_3$ (7.26 and 77.0 ppm). Ultraviolet (UV) spectra were obtained using a Cary Bio 400 spectrophotometer at ambient temperature. Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 Series FT-IR instrument. Absorption bands are reported in wavenumbers (cm^{-1}). Low- and high-resolution mass spectra (LRMS and HRMS) were obtained with electronic or chemical ionization (EI or CI) at The Ohio State University on a Micromass QTOF Electrospray mass spectrometer. Purity of final analogues was judged to be >95% based on their chromatographic homogeneity under two different HPLC conditions.

Iodide (−)-**1** was prepared according to previously published methods,[35] and all intermediates were spectroscopically identical to those previously reported.

Synthesis of *tert*-Butyl Ketone (+)-6. A 15 mL round-bottom flask was charged with diisopropylamine (42 mg, 0.41 mmol, 7.4 equiv—distilled over calcium hydride prior to use) and 2 mL of distilled THF. This solution was cooled to −78 °C, and *n*-butyllithium (250 μL of 1.6 M solution, 0.43 mmol, 7.2 equiv) was added via syringe. Pinacolone (39 mg, 0.39 mmol, 7.0 equiv—dried over potassium carbonate and activated molecular sieves 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, 250 μL) was then added via syringe, and the reaction mixture was allowed to stir for an additional 15 min. A solution of iodide (−)-**5** (25 mg, 0.06 mmol) in 1 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 then warmed to −41 °C in a dry ice/acetonitrile bath where it was allowed to warm to room temperature over the course of 2 h and to stir for an additional 6 h. The resulting yellow solution was 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 (0−20% ethyl acetate/petroleum ether) to give a colorless oil (18 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (t, J = 1.4 Hz, 1H), 4.11 (d, J = 2.0 Hz, 1H), 2.46−1.25 (m, 16H), 1.13 (s, 9H), 1.00 (s, 3H), 0.98−0.96 (d, J = 6.8 Hz, 3H), 0.97−0.93 (t, J = 8 Hz, 9H), 0.59−0.53 (q, J = 7.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 216.1, 160.2, 119.7, 69.0, 55.1, 46.7, 44.1, 36.6, 36.2, 35.8, 35.0, 31.7, 30.7, 26.4, 22.3, 22.0, 18.7, 18.1, 6.9, 4.9. IR (neat): 2956, 1708, 1607, 1456, 1366, 1235, 1143, 1082, 1029, 972, 725 cm^{-1}. $[\alpha]^{25}_D$ +19.4° (c = 2.34, $CHCl_3$). HRMS calcd for $C_{26}H_{48}O_2SiNa$ [M + Na], 443.3321; found, 443.3318.

Synthesis of CD-Ring Ketone (+)-10. A 15 mL round-bottom flask was charged with *tert*-butyl ketone **2** (18 mg, 0.4 mmol) dissolved in 5 mL of distilled THF. Tetrabutylammonium fluoride hydrate (TBAF, 112 mg, 10 equiv) and 4 Å molecular sieves (100 mg) were added to the reaction flask, and this solution was left to stir at reflux for 4 h. Additional portions of TBAF and sieves were added every 4 h until starting material was no longer visible by analytical thin-layer chromatography (TLC). The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove excess TBAF and molecular sieves. This solution was concentrated, and a 10 mL round-bottom flask was charged with the resulting material dissolved in 5 mL of distilled dichloromethane (CH_2Cl_2). To this solution was added 4 Å molecular sieves (20 mg), 4-methylmorpholine-*N*-oxide (NMO, 10 mg, 0.09 mmol, 2 equiv), and a catalytic amount of tetrapropylammonium perruthenate (TPAP). After the solution was stirred for 1 h, TLC showed complete consumption of starting material. The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove TPAP and molecular sieves. This solution was concentrated and purified using silica gel column chromatography (20% ethyl acetate/petroleum ether) to give a colorless oil (9 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29−5.28 (m, J = 1.6 Hz, 1H), 2.87−2.82 (dd, J = 10.6, 6.6 Hz, 1H), 2.47−1.31 (m, 16H), 1.12 (s, 9H), 1.05−1.04 (d, J = 6.8 Hz, 3H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.9, 211.1, 157.8, 120.3, 63.1, 53.8, 44.1, 40.5, 36.5, 36.0, 34.4, 32.9, 27.1, 26.4, 24.0, 21.9, 21.7, 17.25. IR (neat): 2955, 1702, 1461, 1367 cm^{-1}. $[\alpha]^{25}_D$ +15.6° (c = 0.90, $CHCl_3$). HRMS calcd for $C_{20}H_{32}O_2Na$ [M + Na], 327.2300; found, 327.2302.

Synthesis of Analogues 2a and 2b. Anhydrous phosphine oxide (±)-**9** (79 mg, 0.14 mmol, 1.4 equiv) was dissolved in 2.5 mL of distilled THF and cooled to −78 °C. Phenyllithium (88 μL of a 1.7 M solution in cyclohexane−ether, 0.15 mmol, 1.5 equiv) was added dropwise via syringe resulting in a deep red color. This solution was left to stir for 20 min. Anhydrous CD-ring ketone (+)-**10** (31 mg, 0.10 mmol) was dissolved in 1.5 mL of distilled THF and cooled to −78 °C. This solution was then added to the reaction mixture via cannula, and the red color persisted. This solution was stirred at −78 °C in the dark for 7 h at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2 M solution). The product was extracted with ethyl acetate (4 × 60 mL), dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (3−10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2.5 mL of THF, TBAF hydrate (241 mg, 0.92 mmol, 14 equiv), 4 Å molecular sieves (100 mg), and 3 drops of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 8 h. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give **2a** and **2b** (15 mg, 38%) as a mixture of diastereomers (1:3.5), which were separated using normal phase HPLC chromatography (90% ethyl acetate/hexanes, buffered with 1% Et_3N) to give 2 mg (5%, 3% overall) of the natural A-ring isomer **2b**. **2a** (1β, 3α): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.41−6.38 (d, J = 11.2 Hz, 1H), 6.11−6.08 (m, J = 11.2 Hz, 1H), 5.32 (m, 1H), 5.30 (m, 1H), 5.02 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 2.83−2.80 (dm, J = 11.6 Hz, 1H), 2.65−1.33 (m, 19H), 1.13 (s, 9H), 1.03−1.01 (d, J = 6.8 Hz, 3H), 0.67 (s, 3H). $[\alpha]^{25}_D$ = −4.3° (c = 0.08, $CHCl_3$). HRMS calcd for $C_{29}H_{44}O_3Na$ [M + Na], 463.3188; found, 463.3163. **2b** (1α, 3β): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.39−6.36 (d, J = 11.2 Hz, 1H), 6.12−6.09 (m, J = 11.2 Hz, 1H), 5.34−5.33 (t, J = 1.6 Hz, 1H), 5.30−5.29 (t, J = 1.4 Hz, 1H), 5.01 (m, 1H), 4.45 (m, 1H), 4.24 (m, 1H), 2.83−2.79 (dm, J = 12 Hz, 1H), 2.62−2.58 (dm, J = 12.8 Hz, 1H), 2.47−1.33 (m, 18H), 1.12 (s, 9H), 1.03−1.01 (d, J = 6.8 Hz, 3H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 216.1, 159.6, 147.7, 142.6, 132.9, 124.9, 120.3, 116.8, 111.6, 70.6, 66.9, 58.3, 50.1, 45.2, 42.8, 36.6, 36.1, 35.3, 32.7, 29.7, 29.4, 28.8, 26.4, 23.6, 21.9, 21.6, 16.9. IR (neat): 3855, 3752, 3677, 3386, 2924, 2846, 1702, 1654, 1561, 1432, 1362, 1209, 1051, 1010, 846 cm^{-1}. UV (MeOH) λ_{max} 264 nm (ϵ 8157). $[\alpha]^{25}_D$ = 17.2° (c = 0.16, $CHCl_3$). HRMS calcd for $C_{29}H_{44}O_3Na$ [M + Na], 463.3188; found, 463.3175. It was possible to

separate the diastereomers using normal phase HPLC although optimal separation was obtained only by using very small injections (~200 μg or less). Because of the tedious nature of this separation, purification was done so that only the minimum necessary amount of **2b** for biological testing and complete characterization was obtained. The overall yield and complete characterization for the unnatural A-ring isomer **2a** has not yet been determined as unnatural A-ring compounds of this kind are known to be considerably less biologically interesting.[1,38,40]

Synthesis of Analogues 3a and 3b. Anhydrous phosphine oxide (±)-**9** (89 mg, 0.15 mmol, 2 equiv) was dissolved in 2.5 mL of distilled THF and cooled to −78 °C. Phenyllithium (93 μL of a 1.8 M solution in cyclohexane−ether, 0.17 mmol, 2.2 equiv) was added dropwise via syringe resulting in a deep red color. This solution was left to stir for 20 min. Anhydrous CD-ring ketone (+)-**10** (23 mg, 0.08 mmol) was dissolved in 1.5 mL of distilled THF and cooled to −78 °C. This solution was then added to the reaction mixture via cannula, and the red color persisted. This solution was stirred at −78 °C in the dark for 7 h at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2 M solution). The product was extracted with ethyl acetate (4 × 60 mL), dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (3−10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with a portion of this material (27 mg, 0.04 mmol) dissolved in 2 mL of anhydrous pyridine. Hydroxylamine hydrochloride (51 mg, 0.74 mmol, 20 equiv) was added, and the reaction was allowed to stir in the dark at room temperature for 24 h at which point TLC analysis showed complete consumption of starting material and the appearance of a new, more polar product. This material was purified directly using silica gel column chromatography (10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2.5 mL of THF, TBAF hydrate (135 mg, 0.52 mmol, 14 equiv), 4 Å molecular sieves (60 mg), and 3 drops of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 8 h. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to **3a** and **3b** (14 mg, 61%) as a mixture of diastereomers (1:3.5), which were separated using reverse phase HPLC chromatography (38% H_2O/acetonitrile) to give 2.3 mg (16%, 5% overall) of **3a** and 4.0 mg (29%, 10% overall) of **3b**. **3a** (1β, 3α): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.40−6.37 (d, J = 11.2 Hz, 1H), 6.10−6.07 (m, J = 11.2 Hz, 1H), 5.32 (m, 1H), 5.29 (m, 1H), 5.02 (m, 1H), 4.45 (m, 1H), 4.22 (m, 1H), 2.83−2.80 (dm, J = 11.6 Hz, 1H), 2.64−2.60 (dd, J = 12.8 Hz, J = 3.4 Hz, 1H), 2.38−1.24 (m, 18H), 1.11 (s, 9H), 1.03−1.01 (d, J = 6.8 Hz, 3H), 0.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7, 159.7, 147.1, 142.7, 132.7, 125.0, 120.3, 116.8, 112.7, 71.4, 66.7, 58.4, 50.0, 45.4, 42.8, 37.4, 37.2, 35.3, 32.6, 29.4, 28.8, 27.7, 26.1, 24.3, 23.6, 21.4, 17.0. $[\alpha]^{25}_D$ = −3.7 (c = 0.23, $CHCl_3$). HRMS calcd for $C_{29}H_{45}NO_3Na$ [M + Na], 478.3297; found, 478.3336. **3b** (1α, 3β): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.39−6.36 (d, J = 11.6 Hz, 1H), 6.12−6.09 (m, J = 11.6 Hz, 1H), 5.34−5.33 (t, J = 1.6 Hz, 1H), 5.30 (m, 1H), 5.02−5.01 (m, J = 1.6 Hz, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 2.83−2.79 (dm, J = 12 Hz, 1H), 2.63−2.59 (dm, J = 13.6 Hz, 1H), 2.38−1.69 (m, 18H), 1.11 (s, 9H), 1.03−1.02 (d, J = 6.8 Hz, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.7, 159.7, 147.6, 142.7, 132.9, 125.0, 120.3, 116.8, 111.7, 70.7, 66.8, 58.4, 50.0, 45.2, 42.8, 37.4, 37.2, 35.3, 32.6, 29.4, 28.8, 27.7, 26.1, 24.3, 23.6, 21.4, 17.0. IR (neat): 3331, 2955, 2919, 2837, 1661, 1461, 1367, 1349, 1049, 932, 797, 756 cm^{-1}. UV (MeOH) λ_{max} 266 nm (ϵ 8502). $[\alpha]^{25}_D$ = +4.8° (c = 0.33, $CHCl_3$). HRMS calcd for $C_{29}H_{45}NO_3Na$ [M + Na], 478.3297; found, 478.3336.

Synthesis of *tert*-Butyl Methoxy Oxime (+)-7. A 15 mL round-bottom flask was charged with ketone (+)-**3** (125 mg, 0.30 mmol) dissolved in 5 mL of anhydrous pyridine. Methoxylamine hydrochloride (124 mg, 1.49 mmol, 5 equiv) was added, and the reaction was allowed to stir at room temperature for 8 h. The solution was poured into 30 mL of water and extracted using CH_2Cl_2 (3 × 25 mL). This solution was dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (10% ethyl acetate/hexanes) to give a colorless oil (90 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (m, 1H), 4.12 (m, 1H), 3.78 (s, 3H), 2.28−1.22 (m, 16H), 1.10 (s, 9H), 1.02 (s, 3H), 0.98−0.97 (d, J = 6.8 Hz, 3H), 0.98−0.94 (t, J = 7.8 Hz, 9H), 0.60−0.54 (q, J = 8.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 160.3, 128.5, 69.0, 60.9, 55.1, 46.7, 37.3, 37.1, 35.9, 35.0, 31.4, 30.7, 27.8, 26.6, 24.8, 22.0, 18.8, 18.1, 6.9, 4.9. IR (neat): 2954, 1704, 1616, 1463, 1365, 1236, 1147, 1056, 742 cm^{-1}. $[\alpha]^{25}_D$ = +4.4° (c = 0.94, $CHCl_3$). HRMS calcd for $C_{27}H_{52}NO_2Si$ [M + H], 450.3767; found, 450.3763.

Synthesis of CD-Ring Ketone (+)-8. A 15 mL round-bottom flask was charged with *tert*-butyl ketone (+)-**7** (77 mg, 0.17 mmol) dissolved in 20 mL of distilled THF. TBAF (314 mg,1.20 mmol, 7 equiv) and 4 Å molecular sieves (314 mg) were added to the reaction flask, and this solution was left to stir at reflux for 16 h. The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove excess TBAF and molecular sieves. This solution was concentrated and purified using silica gel column chromatography (30% ethyl acetate/hexanes) to give a colorless oil. A 10 mL round-bottom flask was charged with this material dissolved in 5 mL of distilled dichloromethane (CH_2Cl_2). To this solution was added 4 Å molecular sieves (100 mg), NMO (95 mg, 0.82 mmol, 5 equiv), a catalytic amount of TPAP, and 1 drop of triethylamine. After the solution was stirred for 30 min, TLC showed complete consumption of starting material. The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove TPAP and molecular sieves. This solution was concentrated and purified using silica gel column chromatography (15% ethyl acetate/hexanes) to give a yellow oil (38 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28−5.27 (m, J = 1.4 Hz, 1H), 3.77 (s, 3H), 2.86−2.82 (dd, J = 10.6, 6.6 Hz, 1H), 2.47−2.39 (dt, J = 10.8, 1.6 Hz, 1H), 2.30−1.38 (m, 14H), 1.08 (s, 9H), 1.05−1.03 (d, J = 6.8 Hz, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 211.2, 166.6, 157.8, 120.2, 63.2, 60.9, 53.8, 40.5, 37.1, 37.0, 34.4, 32.4, 27.8, 27.0, 26.4, 24.6, 24.0, 21.5, 17.27. IR (neat): 2958, 2871, 1741, 1721, 1465, 1374, 1240, 1055, 886 cm^{-1}. $[\alpha]^{25}_D$ = +7.2° (c = 3.40, $CHCl_3$). HRMS calcd for $C_{21}H_{36}NO_2$ [M + H], 334.2746; found, 334.2744.

Synthesis of Analogues 4a and 4b. Anhydrous phosphine oxide (±)-**9** (101 mg, 0.17 mmol, 1.5 equiv) was dissolved in 2.5 mL of distilled THF and cooled to −78 °C. Phenyllithium (112 μL of a 1.7 M solution in cyclohexane−ether, 0.19 mmol, 1.6 equiv) was added dropwise via syringe resulting in a deep red color. This solution was left to stir for 20 min. Anhydrous CD-ring ketone (+)-**8** (39 mg, 0.12 mmol) was dissolved in 1.5 mL of distilled THF and cooled to −78 °C. This solution was then added to the reaction mixture via cannula, and the red color persisted but lightened to orange after stirring at −78 °C in the dark for 2 h. The reaction was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2 M solution). The product was extracted with ethyl acetate (4 × 60 mL), dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (5% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil (28 mg, 27% yield) and recovered CD-ring ketone (26 mg, 99% yield based on recovered starting material). Attempts to resolve the diastereomers before deprotection proved unsuccessful. The material was carried forward without optimizing the yield in order to determine its biological significance. A 15 mL round-bottom flask was charged with this oil dissolved in 2.5 mL of THF, TBAF hydrate (121 mg, 0.47 mmol, 14 equiv), 4 Å molecular sieves (196 mg), and 2 drops of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 16 h. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give **4a** and **4b** (15 mg, 27%) as a mixture of diastereomers (1:3.5), which were separated using normal phase HPLC chromatography (4%

ethanol/hexanes, buffered with 0.5% Et_3N) to give 4 mg (7%, 2% overall) of the natural A-ring isomer **4b**.

Alternate Synthesis of 4a and 4b. Anhydrous phosphine oxide (±)-**9** (89 mg, 0.15 mmol, 2 equiv) was dissolved in 2.5 mL of distilled THF and cooled to −78 °C. Phenyllithium (93 μL of a 1.8 M solution in cyclohexane−ether, 0.17 mmol, 2.2 equiv) was added dropwise via syringe resulting in a deep red color. This solution was left to stir for 20 min. Anhydrous CD-ring ketone (+)-**10** (23 mg, 0.08 mmol) was dissolved in 1.5 mL of distilled THF and cooled to −78 °C. This solution was then added to the reaction mixture via cannula, and the red color persisted. This solution was stirred at −78 °C in the dark for 7 h at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2 M solution). The product was extracted with ethyl acetate (4 × 60 mL), dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (3−10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with a portion of this material (12 mg, 0.02 mmol) dissolved in 1.5 mL of anhydrous pyridine. Methoxylamine hydrochloride (27 mg, 0.33 mmol, 20 equiv) was added, and the reaction was allowed to stir in the dark at room temperature. Additional portions of methoxylamine were added (60 equiv total), and the reaction was stirred for a total of 36 h. The starting material and the product were very similar in polarity, and TLC analysis was not particularly useful for following the reaction. This material was purified directly using silica gel column chromatography (3% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2 mL of THF, TBAF hydrate (59 mg, 0.22 mmol, 14 equiv), 4 Å molecular sieves (60 mg), and 1 drop of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 24 h. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give **4a** and **4b** (6 mg, 59%) as a mixture of diastereomers (1:3.5), which were separated using reverse phase HPLC chromatography (15% H_2O/acetonitrile) to give 1.4 mg (19%, 6% overall) of **4a** and 2.8 mg (37%, 12% overall) of **4b**. **4a** (1β, 3α): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.41−6.38 (d, J = 11.2 Hz, 1H), 6.11−6.08 (m, J = 11.6 Hz, 1H), 5.32 (m, 1H), 5.29 (t, J = 1.2 Hz, 1H), 5.02 (m, 1H), 4.46−4.44 (m, 1H), 4.24−4.20 (m, 1H), 3.78 (s, 3H), 2.84−2.80 (dm, J = 11.8 Hz, 1H), 2.65−2.60 (dd, J = 13.0 Hz, 3.8 Hz, 1H), 2.38−1.35 (m, 18H), 1.09 (s, 9H), 1.03−1.01 (d, J = 6.8 Hz, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 159.7, 147.1, 142.7, 132.7, 125.0, 120.3, 116.8, 112.5, 71.4, 66.9, 60.9, 58.3, 50.0, 45.2, 42.8, 37.1, 35.3, 32.6, 29.4, 28.8, 27.8, 26.5, 24.6, 23.6, 21.3, 17.0. $[\alpha]^{25}_D$ = 2.4° (c = 0.14. $CHCl_3$). HRMS calcd for $C_{30}H_{48}NO_3$ [M + H], 470.3634; found, 470.3623. **4b** (1α, 3β): ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.39−6.37 (d, J = 11.2 Hz, 1H), 6.12−6.09 (m, J = 11.2 Hz, 1H), 5.34−5.33 (t, J = 1.6 Hz, 1H), 5.29 (t, J = 1.2 Hz, 1H), 5.02 (m, 1H), 4.46−4.43 (m, 1H), 4.26−4.23 (m, 1H), 3.78 (s, 3H), 2.84−2.80 (dm, J = 11.8 Hz, 1H), 2.63−2.58 (dd, J = 13.6 Hz, 3.6 Hz, 1H), 2.38−1.35 (m, 18H), 1.09 (s, 9H), 1.03−1.01 (d, J = 7.2 Hz, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 159.7, 147.6, 142.6, 132.9, 124.9, 120.3, 116.8, 111.7, 70.7, 66.8, 60.9, 58.4, 50.0, 45.2, 42.8, 37.1, 35.3, 32.6, 29.7, 29.4, 28.8, 27.8, 26.5, 24.6, 23.6, 21.3, 16.9. IR (neat): 3331, 2955, 2919, 2849, 1461, 1361, 1049, 885 cm^{-1}. UV (MeOH) λ_{max} 264 nm (ϵ 6923). $[\alpha]^{25}_D$ = +6.0° (c = 0.28, $CHCl_3$). HRMS calcd for $C_{30}H_{48}NO_3$ [M + H], 470.3634; found, 470.3636.

Synthesis of Dimethyl Phenyl Ketone (+)-6′. A 50 mL round-bottom flask was charged with diisopropylamine (167 mg, 1.65 mmol, 7.4 equiv—distilled over calcium hydride prior to use) and 10 mL of distilled THF. This solution was cooled to −78 °C, and *n*-butyllithium (1 mL of 1.6 M solution, 1.60 mmol, 7.2 equiv) was added via syringe. 3-Methyl-3-phenyl-2-butanone (253 mg, 1.56 mmol, 7.0 equiv—prepared according to literature procedure[41]) was dissolved in 5 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. HMPA (500 μL) was then added via syringe, and the reaction mixture was allowed to stir for an additional 15 min. A solution of iodide (−)-**5** (100 mg, 0.22 mmol) in 3 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 1 h, and then, it was allowed to warm to room temperature over the course of 2 h and to stir for an additional 6 h. The resulting yellow solution was quenched with 4 mL of water, extracted with ethyl acetate (3 × 35 mL), dried over $MgSO_4$, concentrated, and purified using silica gel column chromatography (0−20% ethyl acetate/petroleum ether) to give a colorless oil (92 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.23 (m, 5H), 5.16 (t, J = 1.4 Hz, 1H), 4.10 (d, J = 2.0 Hz, 1H), 2.27−1.55 (m, 16H), 1.13 (s, 9H), 1.47 (s, 9H), 0.98−0.94 (t, J = 8 Hz, 9H), 0.92 (s, 3H), 0.90−0.88 (d, J = 6.8 Hz, 3H), 0.59−0.53 (q, J = 7.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.7, 160.0, 148.3, 128.6, 126.7, 126.0, 119.6, 69.0, 55.0, 52.2, 46.6, 37.6, 35.9, 35.7, 34.9, 31.5, 30.7, 25.2, 25.1, 22.4, 22.2, 18.7, 18.0, 6.9, 4.9. IR (neat): 2955, 1742, 1710, 1457, 1372, 1240, 1081, 1030, 734 cm^{-1}. $[\alpha]^{25}_D$ = +20.0° (c = 0.60, $CHCl_3$). HRMS calcd for $C_{31}H_{50}O_2SiNa$ [M + Na], 505.3472; found, 505.3494.

Synthesis of CD-Ring Ketone (+)-10′. A 10 mL round-bottom flask was charged with *tert*-butyl ketone **6′** (46 mg, 0.10 mmol) dissolved in 3 mL of distilled THF. Tetrabutylammonium fluoride (286 μL of a 1.0 M solution, 3 equiv) was added to the reaction flask, and this solution was left to stir at room temperature for 8 h. The reaction mixture was directly purified using silica gel column chromatography (50% ethyl acetate/petroleum ether). The resulting material was dissolved in 5 mL of distilled dichloromethane (CH_2Cl_2). To this solution was added 4 Å molecular sieves (20 mg), NMO (15 mg, 0.13 mmol, 2 equiv), and a catalytic amount of TPAP. After the solution was stirred for 1 h, TLC showed complete consumption of starting material. The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove TPAP and molecular sieves. This solution was concentrated and purified using silica gel column chromatography (20% ethyl acetate/petroleum ether) to give a colorless oil (14 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.23 (m, 5H), 5.18−5.17 (t, J = 1.2 Hz, 1H), 2.82−2.78 (dd, J = 10.8, 6.4 Hz, 1H), 2.43−2.36 (ddm, J = 15.8, 10.6 Hz, 1H), 2.29−2.25 (m, 2H), 2.19−2.15 (t, J = 7.0 Hz, 2H), 2.12−1.68 (m, 10H), 1.47 (s, 6H), 0.97−0.95 (d, J = 6.8 Hz, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 213.0, 211.1, 157.6, 144.0, 128.6, 126.8, 126.0, 120.2, 63.1, 53.7, 52.2, 40.5, 37.4, 35.7, 34.3, 32.6, 27.0, 25.1, 25.1, 24.0, 22.3, 21.6, 17.2. IR (neat): 2965, 1712, 1496, 1448, 1373, 1242, 1047 cm^{-1}. $[\alpha]^{25}_D$ +20.1° (c = 0.1.60, $CHCl_3$). HRMS calcd for $C_{25}H_{34}O_2Na$ [M + Na], 389.2451; found, 389.2465.

Synthesis of 4a′ and 4b′. Anhydrous phosphine oxide (±)-**9** (53 mg, 0.09 mmol, 1.9 equiv) was dissolved in 1.5 mL of distilled THF and cooled to −78 °C. Phenyllithium (45 μL of a 2.0 M solution in cyclohexane−ether, 0.09 mmol, 1.9 equiv) was added dropwise via syringe resulting in a deep red color. This solution was left to stir for 20 min. Anhydrous CD-ring ketone (+)-**10′** (18 mg, 0.05 mmol) was dissolved in 1.0 mL of distilled THF and cooled to −78 °C. This solution was then added to the reaction mixture via cannula, and the red color persisted. This solution was stirred at −78 °C in the dark for 4 h at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2 M solution). The product was extracted with ethyl acetate (4 × 30 mL), dried using $MgSO_4$, filtered, concentrated, and purified using silica gel column chromatography (3−10% ethyl acetate/petroleum ether buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with a portion of this material (12 mg, 0.02 mmol) dissolved in 1.5 mL of anhydrous pyridine. Methoxylamine hydrochloride (27 mg, 0.33 mmol, 20 equiv) was added, and the reaction was allowed to stir in the dark at room temperature. Additional portions of methoxylamine were added (100 equiv total), and the reaction was stirred for a total of 36 h. The starting material and the product were very similar in polarity and TLC analysis was not particularly useful for following the

reaction. This material was purified directly using silica gel column chromatography (3% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2 mL of THF, TBAF (112 μL of a 1.0 M solution, 0.11 mmol, 7 equiv), and 1 drop of Et_3N. This solution was left to stir in the dark at room temperature for 24 h. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give **4a′** and **4b′** (6 mg, 25%) as a mixture of diastereomers (1:2), which were separated using reverse phase HPLC chromatography (15% H_2O/acetonitrile) to give 1.5 mg (18%, 6% overall) of **4a′** and 2.6 mg (22%, 8% overall) of **4b′**. **4a′** (1β, 3α): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30−7.18 (m, 5H), 6.41−6.38 (d, J = 11.6 Hz, 1H), 6.10−6.08 (m, J = 11.2 Hz, 1H), 5.34 (m, 1H), 5.16 (m, 1H), 5.04 (m, 1H), 4.46 (m, 1H), 4.22 (m, 1H), 3.88 (s, 3H), 2.83−2.79 (dm, J = 13.2 Hz, 1H), 2.65−2.60 (m, 1H), 2.33−1.55 (m, 18H), 1.46 (s, 6H), 0.91−0.90 (d, J = 6.8 Hz, 3H), 0.60 (s, 3H). HRMS calcd for $C_{35}H_{49}NO_3Na$ [M + Na], 554.3605; found, 554.3655. **4b′** (1α, 3β): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30−7.18 (m, 5H), 6.39−6.36 (d, J = 11.2 Hz, 1H), 6.11−6.09 (m, J = 11.6 Hz, 1H), 5.36 (m, 1H), 5.16 (m, 1H), 5.03 (m, 1H), 4.45 (m, 1H), 4.24 (m, 1H), 3.88 (s, 3H), 2.82−2.79 (dm, J = 12.8 Hz, 1H), 2.62−2.59 (dm, J = 13.6 Hz, 1H), 2.35−1.63 (m, 18H), 1.46 (s, 6H), 0.91−0.90 (d, J = 6.8 Hz, 3H), 0.61 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 159.6, 147.7, 146.2, 142.7, 132.9, 128.2, 126.3, 126.2, 125.0, 120.2, 116.8, 111.5, 70.7, 66.9, 61.3, 58.4, 50.0, 45.2, 45.1, 42.9, 37.0, 35.3, 32.5, 29.3, 28.8, 28.5, 26.8, 26.8, 24.6, 23.6, 21.3, 16.9. IR (neat): 3363, 2919, 2849, 1458, 1053. $[\alpha]^{25}_D = +7.5°$ (c = 0.24, $CHCl_3$). HRMS calcd for $C_{35}H_{49}NO_3Na$ [M + Na], 554.3605; found, 554.3604.

In Vitro Testing Materials. Murine keratinocyte cell line PE was kindly provided by Dr. James E. Strickland, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute. Chosen for its particular sensitivity to the induction of ornithine decarboxylase (ODC) activity by the extensively characterized tumor promoter TPA, cell line PE was derived from papilloma-induced in female SENCAR mice by a standard skin initiation/promotion protocol.[42] PE cell culture medium consisted of Eagle's minimum essential medium without calcium chloride (Whittake Bioproducts, Walkersville, MA) supplemented with 8% chelexed fetal calf serum and 1% antibiotic−antimycotic (Gibco BRL) and the addition of $CaCl_2$ to 0.05 mM Ca^{2+}.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma Chemical Co. (St. Louis, MO), and TPA was supplied by L. C. Services (Woburn, MA). l-[^{14}C]Ornithine (56 μCi/mol) was from Amersham/Searle Corp. (Arlington Heights, IL). Chemical solvents used in all assays of biological activity were of the highest grade commercially available.

Growth Inhibition. Growth curves for PE cells treated with calcitriol and the analogues reported herein were generated by assay for the reduction of the tetrazolium-based compound MTT.[43] A mitochondrial dehydrogenase reduces MTT to a blue formazan product with an absorbance maximum of 505 nm in dimethyl sulfoxide (DMSO); the number of viable cells can thus be determined spectrophotometrically. PE cells were seeded at a density of 5000 cells/well in 50 μL of the medium into 96 well microtiter plates. Twelve hours later, the medium was removed, and cells were treated with 100 μL of fresh medium into which the appropriate amount of calcitriol or analogue dissolved in DMSO had been added, with the concentration of DMSO held constant at 0.1%. The plates were fed once at 48 h, with the readdition of the vitamin D_3 analogues at this time. At 24 h intervals following the initial treatment of the cells with compounds, 0.1 mg (50 μL of a 2 mg/mL solution) of MTT was added to each well. After 4 h, the MTT was removed and DMSO was added to dissolve the blue formazan dye. Using a microtiter plate reader, the A_{505} was then determined and cell number was calculated from blank-subtracted absorbance values. Results from the MTT assay for the inhibition if cell growth were independently confirmed by treating 100 cm^2 dishes of cells in an analogous manner for 96 h, whereupon the cells were harvested by trypsinization and counted. Furthermore, the viability of the cells treated with calcitriol or analogues was determined to be identical to control cells at 96 h by trypan blue exclusion.

Determination of Urinary Calcium Levels. Male F344 rats (150 g) were housed individually in glass metabolism cages and received food and water ad libitum. After several days acclimation, rats received 1 μg/kg body weight of test compound per os for 7 consecutive days in 150 μL of propylene glycol/0.05 M Na_2HPO_4 (80:20). Urine samples, which were collected on ice, were centrifuged at 650*g* for 10 min, adjusted to pH 6.0 as necessary, and assayed for calcium content spectrophotometrically at 575 nm using reagents and standards from Sigma Calcium Kit no. 587.

Acknowledgment. We thank the NIH for support of this work (CA 44530 to G.H.P. and T.W.K. and DK 50583 to S.P.).

References

(1) Bouillon, R.; Okamura, W. H.; Norman, A. W. Function Relationships in the Vitamin D Endocrine System. *Endocrine Rev.* **1995**, *16*, 200−257.
(2) Guyton, K. Z.; Kensler, T. W.; Posner, G. H. Cancer Chemoprevention Using Natural Vitamin D and Synthetic Analogues. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 421−442.
(3) Konety, B. R.; Johnson, C. S.; Trump, D. L.; Getzenberg, R. H. *Semin. Urol. Oncol.* **1999**, *17*, 77−84.
(4) Feldman, D.; Glorieux, F.; J. W., P. *Vitamin D*; Academic Press: San Diego, CA, 1997.
(5) Holick, M. F. *Vitamin D: Molecular Biology, Physiology, and Clinical Applications*; Humana Press: Totowa, NJ, 1999.
(6) Kabat, M. M.; Burger, W.; Guggino, S.; Hennessy, B.; Iacobelli, J. A.; Takeuchi, K.; Uskokovic, M. R. Total Synthesis of 25-Hydroxy-16,23E-Diene Vitamin D_3 and 1α,25-Dihydroxy-16,23E-Diene Vitamin D_3: Separation of Genomic and Nongenomic Vitamin D Activities. *Bioorg. Med. Chem.* **1998**, *6*, 2051−2059.
(7) Hansen, C. M.; Hamberg, K. J.; Binderup, E.; Binderup, L. Seocalcitol (EB 1089): A Vitamin D Analogue of Anti-cancer Potential. Background, Design, Synthesis, Pre-clinical and Clincial Evaluation. *Curr. Pharm. Des.* **2000**, *6*, 803.
(8) Posner, G. H.; Crawford, K. R.; Peleg, S.; Welsh, J.-E.; Romu, S.; Gewirtz, D. A.; Gupta, M. S.; Dolan, P.; Kensler, T. W. A Non-Calcemic Sulfone Version of the Vitamin D3 Analogue Seocalcitol (EB1089). *Bioorg. Med. Chem.* **2001**, *9*, 2365−2371.
(9) Uskokovic, M. R.; Norman, A. W.; Manchand, P. S.; Studzinski, G. P.; Campbell, M. J.; Koeffler, H. P.; Takeuchi, A.; Siu-Caldera, M.-L.; Rao, D. S.; Reddy, G. S. Highly Active Analogues of 1α,25-Dihydroxyvitamin D_3 That Resist Metabolism Through C-24 Oxidation and C-3 Epimerization Pathways. *Steroids* **2001**, *66*, 463−471.
(10) Lee, N. E.; Williard, P. G.; Brown, A. J.; Campbell, M. J.; Koeffler, H. P.; Peleg, S.; Rao, D. S.; Reddy, G. S. Synthesis and Biological Activities of the Two C(23) Epimers of 1α,23,25-Trihydroxy-24-Oxo-19-Nor-Vitamin D_3: Novel Analogues of 1α,23(S),25-Trihydroxy-24-Oxo-Vitamin D_3, a Natural Metabolite of 1α,25-Dihyroxyvitamin D_3. *Steroids* **2000**, *65*, 252−265.
(11) Posner, G. H.; Lee, J. K.; White, M. C.; Hutchings, R. H.; Dai, H.; Kachinski, J. C.; Dolan, D.; Kensler, T. W. Antiproliferative Hybrid Analogues of 1α,25-Dihydroxyvitamin D_3: Design, Synthesis, and Preliminary Biological Evaluation. *J. Org. Chem.* **1997**, *62*, 3299−3314.
(12) Uskokovic, M. R.; Studzinski, G. P.; Gardner, J. P.; Reddy, S. G.; Campbell, M. J.; Koeffler, H. P. The 16-Ene Vitamin D Analogues. *Curr. Pharm. Des.* **1997**, *3*, 99−123.
(13) Salituro, G. M.; Townsend, C. A. Total Syntheses of (−)-Nocardicins A−G: A Biogenetic Approach. *J. Am. Chem. Soc.* **1990**, *112*, 760−770.
(14) Hong, C. Y.; Kim, Y. K.; Lee, Y. H.; Kwak, J. H. Methyloxime-Substituted Aminopyrrolidine: A New Surrogate for 7-Basic Group of Quinolone. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 221−226.
(15) Xu, R.; Sim, M.-K.; Go, M.-L. Synthesis and Pharmacological Characterization of *O*-Alkynyloximes of Tropinone and *N*-Methylpiperidinone as Muscarinic Agonists. *J. Med. Chem.* **1998**, *41*, 3220−3231.
(16) Frederickson, M.; Parker, E. J.; Hawkins, A. R.; Coggins, J. R.; Abell, C. Selective Inhibition of Type II Dehydroquinases. *J. Org. Chem.* **1999**, *64*, 2612−2613.

(17) Knutsen, L. J. S.; Anderson, K. E.; Lau, J.; Lundt, B. F.; Henry, R. F.; Morton, H. E.; Naerum, L.; Petersen, H.; Stephensen, H.; Suzdak, P. D.; Swedberg, M. D. B.; Thomsen, C.; Sorensen, P. O. Synthesis of Novel GABA Uptake Inhibitors. 3. Diaryloxime and Diarylvinyl Ether Derivatives of Nipecotic Acid and Guvacine as Anticonvulsant Agents. *J. Med. Chem.* **1999**, *42*, 3447−3462.

(18) Lin, S.-W.; Yang, D.-Y. Inhibition of 4-Hydroxyphenylpyruvate Dioxygenase by Sethoxydim, a Potent Inhibitor of Acetyl-Cuenzyme A Carboxylase. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 551−554.

(19) Gallardo, T.; Zafra-Polo, M. C.; Tormo, J. R.; González, M. C.; Franck, X.; Estornell, E.; Cortes, D. Semisynthesis of Antitumoral Acetogenins: SAR of Functionalized Alkyl-Chain Bis-Tetrahydrofuranic Acetogenins, Specific Inhibitors of Mitochondrial Complex I. *J. Med. Chem.* **2000**, *43*, 4793−4800.

(20) Tanaka, K.; Katsurada, M.; Ohno, F.; Shiga, Y.; Oda, M. Practical Asymmetric Synthesis of (S)-MA20565, a Wide-Spectrum Agricultural Fungicide. *J. Org. Chem.* **2000**, *65*, 432−437.

(21) Yanagisawa, H.; Takamura, M.; Yamada, E.; Fujita, S.; Fujiwara, T.; Yachi, M.; Isobe, A.; Hagisawa, Y. Novel Oximes Having 5-Benzyl-2,4-thiazolidinedione as Antihyperglycemic Agents: Synthesis and Structure−Activity Relationship. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 373−375.

(22) Dallavalle, S.; Ferrari, A.; Biasotti, B.; Merlini, L.; Penco, S.; Gallo, G.; Marzi, M.; Tinti, M. O.; Martinellii, R.; Pisano, C.; Carminati, P.; Carenini, N.; Beretta, G.; Perego, P.; De Cesare, M.; Pratesi, G.; Zunino, F. Novel 7-Oximinomethyl Derivatives of Camptothecin with Potent in Vitro and in Vivo Antitumor Activity. *J. Med. Chem.* **2001**, *44*, 3264−3274.

(23) Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. M. Discovery of 4-[(Z)−(4-Bromophenyl-(ethoximino)methyl]-1′-[2,4-dimethyl-3-pyridinyl)-carbonyl]-4′-methyl-1,4′-bipyridine N-Oxide (SCH 351125): An Orally Bioavailable Human CCR5 Anatagonist for Treatment of HIV Infection. *J. Med. Chem.* **2001**, *44*, 3339−3342.

(24) Rodríguez, J.; Nunez, L.; Peixinho, S.; Jiménez, C. Isolation and Synthesis of the First Natural 6-Hydroximino 4-en-3-one- Steroids from the Sponges *Cinachyretta* Spp. *Tetrahedron Lett.* **1997**, *38*, 1833−1836.

(25) Ganem, B.; Dong, Y.; Zheng, Y. F.; Prestwich, G. D. Amidrazone and Amidoxime Inhibitors of Squalene Hopene Cyclase. *J. Org. Chem.* **1999**, *64*, 5441−5446.

(26) Hejaz, H. A. M.; Purohit, A.; Mahon, M. F.; Reed, M. J.; Potter, B. V. L. Synthesis and Biological Activity of the Superestrogen (*E*)-17-Oximino-3-*O*-sulfamoyl-1,3,5(10)-estratriene: X-ray Crystal Structure of (*E*)-17-Oximino-3-hydroxy-1,3,5(10)-estratriene. *J. Med. Chem.* **1999**, *42*, 3188−3192.

(27) Cerri, A.; Almirante, N.; Barassi, P.; Benicchio, A.; Fedrizzi, G.; Ferrari, P.; Micheletti, R.; Quadri, L.; Ragg, E.; Rossi, R.; Santagostino, M.; Schiavone, A.; Serra, F.; Zappavigna, M. P.; Melloni, P. 17*β*-*O*-Aminoalkyloximes of 5*β*-Androstane-3*β*,14*β*-diol with Digitalis-like Activity: Synthesis, Cardiotonic Activity, Structure−Activity Relationships, and Molecular Modeling of the Na^+, K^+-ATPase Receptor. *J. Med. Chem.* **2000**, *43*, 2332−2349.

(28) Hartmann, R. W.; Hector, M.; Haidar, S.; Ehmer, P. B.; Reichert, W.; Jose, J. Synthesis and Evaluation of Novel Steroidal Oxime Inhibitors of P450 17 (17α-Hydroxylase/C17−20-Lyase) and 5α-Reductase Types 1 and 2. *J. Med. Chem.* **2000**, *43*, 4266−4277.

(29) Haidar, S.; Klein, C. D. P.; Hartmann, R. W. Synthesis and Evaluation of Steroidal Hydroxamic Acids as Inhibitors of P450 17 (17α-Hydroxylase/C17−20-Lyase). *Arch. Pharm. Med. Chem.* **2001**, *334*, 138−140.

(30) Hawkes, G. E.; Herwig, K.; Roberts, J. D. Nuclear Magnetic Resonance Spectroscopy. Use of ^{13}C Spectra to Establish Configuration of Oximes. *J. Org. Chem.* **1974**, *39*, 1017−1028.

(31) Posner, G. H.; Nelson, T. D.; Guyton, K. Z.; Kensler, T. W. New Vitamin D_3 Derivatives With Unexpected Antiproliferative Activity. *J. Med. Chem.* **1992**, *35*, 3280−3287.

(32) Ishida, H.; Shimizu, M.; Yamamoto, K.; Iwasaki, Y. Syntheses of 1-Alkyl-1,25-Dihydroxyvitamin D_3. *J. Org. Chem.* **1995**, *60*, 1828.

(33) Eguchi, T.; Ikekawa, N. Conformational Analysis of 1α,25-Dihydroxyvitamin D_3 by Nuclear Magnetic Resonance. *Bioorg. Chem.* **1990**, *18*, 19.

(34) Anet, F. A. L. The Use of Remote Deuteration for the Determination of Coupling Constants and Conformational Equilibria in Cyclohexane Derivatives. *J. Med. Chem. Soc.* **1962**, *84*, 1053.

(35) Posner, G. H.; Lee, J. K.; Wang, Q.; Peleg, S.; Burke, M.; Brem, H.; Dolan, P.; Kensler, T. W. Noncalcemic, Antiproliferative, Transcriptionally Active, 24-Fluorinated Hybrid Analogues of the Hormone 1α,25-Dihydroxyvitamin D_3. Synthesis and Preliminary Biological Evaluation. *J. Org. Chem.* **1998**, *41*, 3008−3014.

(36) Peleg, S.; Liu, Y. Y.; Reddy, S.; Horst, R. L.; White, M. C.; Posner, G. H. A 20-Epi Side Chain Restores Growth-Regulatory and Transcriptional Activities of an A Ring-Modified Hybrid Analogue of 1α,25-Dihydroxyvitamin D_3 Without Increasing Its Affinity to the Vitamin D Receptor. *J. Cell. Biochem.* **1996**, *63*, 149−161.

(37) Peleg, S.; Nguyen, C.; Woodard, B. T.; Lee, J.-K.; Posner, G. H. Differential Use of Transcription Activation Function 2 Domain of the Vitamin D Receptor by 1α,25-Dihydroxyvitamin D_3 and Its A Ring-Modified Analogues. *Mol. Endocrinol.* **1998**, 525−535.

(38) Posner, G. H.; Wang, Q.; Han, G.; Lee, J. K.; Crawford, K.; Zand, S.; Brem, H.; Peleg, S.; Dolan, P.; Kensler, T. W. Conceptually New Sulfone Analogues of the Hormone 1α,25-Dihydroxyvitamin D_3: Synthesis and Preliminary Biological Evaluation. *J. Med. Chem.* **1999**, *42*, 3425−3435.

(39) Jones, G.; Strugnell, S. A.; DeLuca, H. F. Current Understanding of the Molecular Actions of Vitamin D. *Physiol. Rev.* **1998**, *78*, 1193−1231.

(40) Posner, G. H.; Crawford, K.; Siu-Caldera, M.-L.; Reddy, G. S.; Sarabia, S. F.; Feldman, D.; van Etten, E.; Mathieu, C.; Gennaro, L.; Vouros, P.; Peleg, S.; Dolan, P.; Kensler, T. W. Conceptually New 20-*epi*-22-Oxa Sulfone Analogues of the Hormone 1α,25-Dihydroxyvitamin D_3: Synthesis and Biological Evaluation. *J. Med. Chem.* **2000**, *43*, 3581−3586.

(41) Aranda, A.; Diaz, A.; Diezbarra, E.; Delaholz, A.; Moreno, A.; Sanchezverdu, P. Solid Liquid Phase-Transfer Catalysis without Solvent−Selective Monoalkylation and Dialkylation of Benzyl Methyl Ketone. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2427.

(42) Yuspa, S. H.; Morgan, D.; Lichti, U.; Spangler, E. F.; Michael, D.; Kilkenny, A.; Hennings, H. Cultivation and Characterization of Cells Derived from Mouse Skin Papillomas Induced by an Initiation-Promotion Protocol. *Carcinogenesis* **1986**, *7*, 949−958.

(43) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a Tetrazolium-Based Semi-Automated Colorimetric Assay: Assessment of Chemosensitivity Testing. *Cancer Res.* **1987**, *47*, 936−942.

JM010560O