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Synthesis and antitumor activity of C-9 epimers of the tetrahydrofuran containing acetogenin 4-deoxyannoreticuin

Feng Wang, Akira Kawamura, David R. Mootoo *

Department of Chemistry, Hunter College, 695 Park Avenue, New York, NY 10065, USA
The Graduate Center, CUNY, 365 5th Avenue, New York, NY 10016, USA

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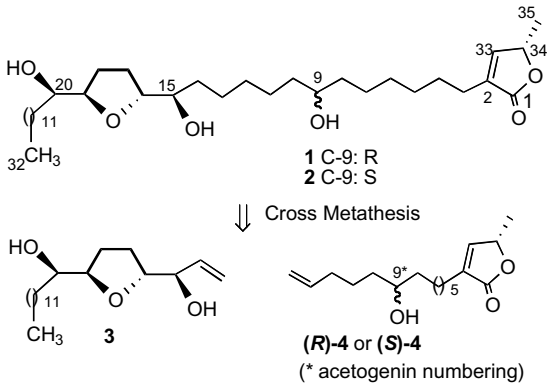
ABSTRACT

A highly convergent synthesis of mono-tetrahydrofuran (THF) containing acetogenins, that is based on the cross-metathesis of THF and butenolide alkene precursors, was developed. This methodology was applied to the epimers of the C-9 alcohol of 4-deoxyannoreticuin, in an attempt to assign the configuration at this position in the naturally occurring material. Unfortunately, identification of one or the other epimeric structures with the natural product was not possible because of the closeness of the physical data for all three compounds. Both C-9 epimeric analogues showed similar cytotoxicity in the low micromolar range, against two human tumor cell lines PC-3 (prostate) and Jurkat (T-cell leukemia). This result contrasts to previous studies on closely related THF acetogenins, wherein configurational variation at analogous carbinol centers resulted in a significant effect on antitumor activity.

1. Introduction

The tetrahydrofuran (THF) containing acetogenin family of natural products, of which over 400 have been isolated, are known for their cytotoxic, antitumoral, insecticidal, and immunosuppresive activities.[1] Three major subtypes, depending on the number and connectivity of the THF rings, have been identified: mono-THF's, adjacently linked bis-THFs, and non-adjacently linked bis-THFs. The cellular target is believed to be the reduced nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase in complex **I**, a membrane bound protein of the mitochondrial electron transport system.[2] Inhibition of electron transfer is believed to disrupt ATP production, which may lead to cell death via a necrosis mechanism. More recent studies suggest that apoptotic pathways could also be involved and may account for the high potency.[3] The ubiquinone-linked NADH oxidase that is peculiar to the plasma membrane of cancerous cells has also been suggested as a site of action, and this may explain the activity of the acetogenins against multidrug resistant tumors.[4] The generally high cytotoxicity of these agents presents a challenge to their progress as clinical agents, and in this vein less active analogues might be better suited for lead development. Synthetic studies on the THF acetogenins are relevant to bio-mechanistic investigations[5] and have also been valuable for structure determination, in particular with regard to stereochemical assignment.[6]

Against this backdrop we were attracted to 4-deoxyannoreticuin, a relatively simple mono-THF acetogenin with low micromolar level antitumor activity, because the stereochemistry was not completely assigned.[7] We were particularly interested in a synthetic plan that would be practical for assigning an unambiguous structure to 4-deoxyannoreticuin and in the longer term would be applicable to other mono-THF acetogenin congeners. The gross structure of 4-deoxyannoreticuin is represented as in **1** or **2** but the configuration at C-9 was not determined (Scheme 1). The relative stereochemistry of the C15–C20 portion was deduced as the threo–trans–threo motif on the basis of the known ^{1}H and ^{13}C NMR trends.[8] The absolute stereochemistry of the THF core was as-



Scheme 1. Retrosynthesis for 4-deoxyannoreticuin.

* Corresponding author. Contact Address: Department of Chemistry, Hunter College, 695 Park Avenue, New York, 10065, USA. Tel.: +1 212 772 4356; fax: +1 212 772 5332.
E-mail address: dmootoo@hunter.cuny.edu (D.R. Mootoo).

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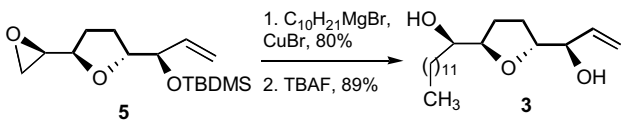
signed as 15R, 16R, 19R, 20R because this configuration was determined for the analogous segment in 2,4-*trans*-squamoxinone, a co-isolate from the same plant material, and several other mono-THF containing acetogenins.[7] It was also assumed that the configuration at C-34 is (*S*) by analogy with all the other acetogenins for which this center has been determined.[9] In order to establish the configuration at C-9, we set as synthetic goals the C-9 epimeric structures **1** and **2**, with the plan of matching one or the other to the natural product on the basis of its physical properties. Accordingly, a modular synthesis in which a fixed THF segment **3** could be paired with epimeric-C-9-hydroxy-butenolide components (*R*)-**4** and (*S*)-**4** (or appropriately protected alcohol derivatives), via an olefin cross metathesis (CM), was conceived.[10,11]

2. Results and discussion

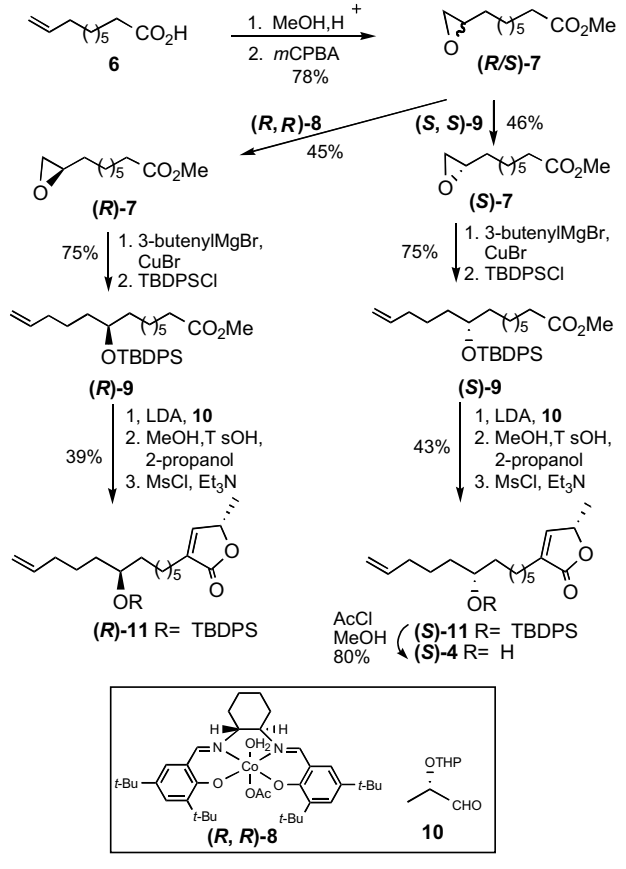
The synthesis of the THF segment followed our earlier work on the analogue of **3** that contained a hydrocarbon chain with two carbons less.[10b] Thus, the copper (I)-mediated reaction of undecylmagnesium bromide with the known epoxide **5**, followed by desilylation of the product provided diol **3** (Scheme 2).

The epimeric butenolide segments (*R*)-**4** and (*S*)-**4**, originated from the racemic mixture of epoxided rac-**7** are derived from the reaction of methyl dec-9-enoate with mCPBA (Scheme 3). Treatment of rac-**7** under Jacobsen's Hydrolytic Kinetic Resolution (HKR) conditions using **(*R*, *R*)-8**, or its enantiomer gave either (*R*)-**7** or (*S*)-**7** in 45% and 46% yields, respectively.[12] Epoxides (*R*)-**7** and (*S*)-**7** were next converted to the silyl ethers (*R*)-**9** and (*S*)-**9,** respectively, via a two-step epoxide opening-alcohol silylation sequence. The ee of these products were deduced to be over 95% by ^{1}H NMR analysis of their Mosher ester derivatives (Supplementary Information).[13] Silyl ethers (*R*)-**9** and (*S*)-**9** were individually partnered with aldehyde **10** and forwarded into a known aldol reaction-elimination protocol to give butenolide alkenes (*R*)-**11** and (*S*)-**11,** respectively, in 39% and 43% overall yield from (*R*)-**9** and (*S*)-**9**.[14] To compare the use of protected versus deprotected alcohol butenolide partners in the CM and subsequent reactions (vide infra), one of the silyl ethers (*S*)-**11** was converted to the derived alcohol (*S*)-**4**.

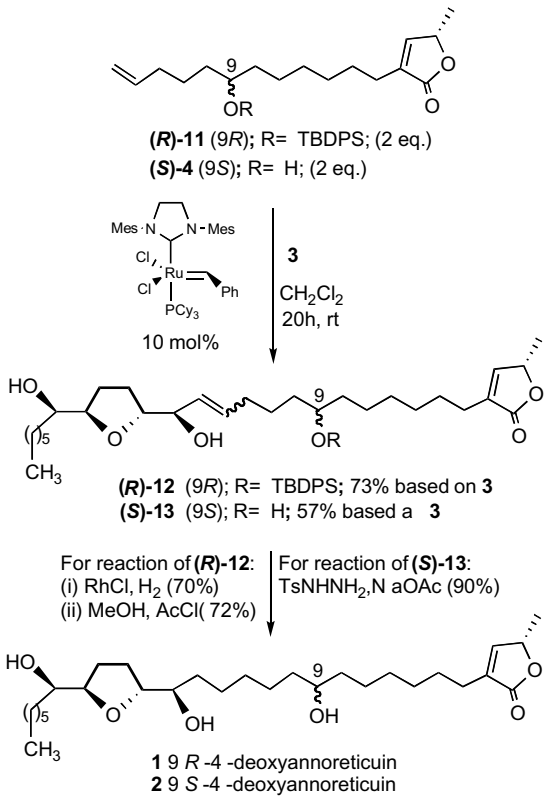
The CM and subsequent steps were first evaluated for TBDPS-protected butenolide (*R*)-**11** (Scheme 4). The optimal CM conditions were found to be methylene chloride as the solvent, a 1:2 molar ratio of **3**:(*R*)-**11**, 10 mole% Grubbs II catalyst, at room temperature for approximately 20 h. The desired product (*R*)-**12** was obtained in 73% isolated yield based on THF segment **3**. The homodimer of (*R*)-**11** was also isolated in 20% yield together with approximately 15% of unreacted (*R*)-**11**. Selective hydrogenation of the isolated alkene in (*R*)-**12** using $RhCl(PPh_3)_3$ as a catalyst provided an inseparable 7:1 mixture of the desired hydrogenated product and the completely hydrogenated material in 70% combined yield. Exposure of this mixture to methanolic HCl led to 9*R*-4-deoxyannoreticuin (**1**) that contained approximately 10% of the corresponding over-reduced product. For the synthesis of 9*S*-4-deoxyannoreticuin (**2**), the CM reaction was performed with the desilylated butenolide (*S*)-**4** thereby obviating the desilylation step in a more advanced THF-butenolide synthetic intermediate. Accordingly, application of the CM conditions to **3** and (*S*)-**4** gave



Scheme 2. Synthesis of THF segment.



Scheme 3. Synthesis of butenolide segments.



Scheme 4. Cross metathesis and final products.

(*S*)-**13** in 57% isolated yield based on **3**. Given the difficulty in controlling the $RhCl(PPh_3)_3$-catalyzed hydrogenation of (*R*)-**12**, the diimide reduction of (*S*)-**13** was explored and found to be superior, giving 9*S*-4-deoxyannoreticuin (**2**) in 90% yield with no evidence of the over-reduced product.

In the absence of an actual sample of 4-deoxyannoreticuin we attempted to assign the configuration at C-9 in the natural product by comparison of the NMR data for synthetic **1** and **2** with the reported listings for natural 4-deoxyannoreticuin.[7] All three sets of data were essentially identical, which corroborated the gross structure assigned to the natural product, but at the same time made assignment of the C-9 configuration impossible. This scenario was not completely surprising given the close similarity (albeit noticeably different) of the NMR data for the analogous epimers of corossolin, the C-10 regioisomer of 4-deoxyannoreticuin.[15,16] Similar observations have been made in a recent more rigorous investigation on acetogenin diastereomers that vary with respect to the configuration at remote stereogenic centers.[6e] Examination of optical rotations were also inconclusive because the values obtained for **1** and **2** were appreciably different from that reported for the natural product, ($[\alpha]_D$ = +19°, +25° and +6.8° for **1**, **2,** and natural 4-deoxyannoreticuin, respectively). Parenthetically, the optical rotations for **1** and **2** were essentially identical to the values observed for the respective C-10 epimers of corossolin.[15]

In the case of corossolin[15], the cytotoxic activities of the C-10 epimers were significantly different. Therefore, we next attempted to identify **1** or **2** with the natural product by comparing their cytotoxicity against PC-3 (human prostate) with the expectation that the activity of one or the other would more closely match the reported data for the natural product. The GI_{50} values for **1** and **2** were found to be approximately 15 and 10 μg/mL in a 3-day MTS assay. The ED_{50} reported for the natural sample is 2.7 μg/mL in a 7-day MTT assay.[7] Thus, preliminary biological evaluation was also not structurally insightful. Epimers **1** and **2** also showed similar activity against Jurkat cells (human T-cell leukemia), with GI_{50} values in the range of 5–10 μg/mL. These data suggest that the notion that a change in the configuration at such remote carbinol centers leads to significant difference in cytotoxic activity may hold only for certain cell lines and is not general.

3. Conclusion

In conclusion we have developed a highly convergent synthesis for mono-THF containing acetogenins that is based on the CM coupling of THF and butenolide components. This methodology was applied to the epimeric analogues of the natural product 4-deoxyannoreticuin, in an attempt to resolve the unassigned configuration at C-9. Unfortunately, identification of one or the other epimeric structures with the natural product was not possible because of the closeness of the physical data for all three compounds, illustrating the limitation of this strategy for the assignment of stereochemistry at remote stereogenic centers. In contrast to observations made for closely related THF acetogenins, the cytotoxicity of the epimeric analogues of 4- deoxyannoreticuin was found to be very similar.

4. Experimental

4.1. Chemistry

Solvents were purified by standard procedures or used from commercial sources as appropriate. Petroleum ether refers to the fraction of petroleum ether boiling between 40 and 60 °C. Unless otherwise stated, thin layer chromatography (TLC) was done on 0.25 mm thick precoated silica gel 60 (HF-254, Whatman) aluminium sheets and flash column chromatography (FCC) was performed using Kieselgel 60 (32–63 mesh, Scientific Adsorbents). Elution for FCC usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. Chromatograms were observed under UV (short and long wavelength) light, and/or were visualized by heating plates that were dipped in a solution of ammonium (VI) molybdate tetrahydrate (12.5 g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Optical rotations ($[\alpha]_D$ were recorded using a Rudolph Autopol III polarimeter, which has a thermally jacketed 10 cm cell (path length of 1 dm), and are given in units of 10^{-1} deg cm^2 g at 589 nm (sodium D-line). NMR spectra were recorded using either Varian Unity Plus 500 or Bruker Ultra Shield instruments (^1H and ^{13}C; 500 and 125 MHz, respectively). Spectra were recorded in $CDCl_3$ solutions with residual $CHCl_3$ as internal standard (δ_H 7.27 and δ_C 77.0 ppm). Chemical shifts are quoted in ppm relative to tetramethysilane (δ_H 0.00), and coupling constants (J) are given in Hertz (Hz). First-order approximations are employed throughout. High-resolution mass spectrometry was performed on an Ultima Micromass Q-Tof instrument at the Mass Spectrometry Laboratory of the University of Illinois, Urbana-Champaign.

4.1.1. (2*R*,5*R*,1′*R*,1″*R*)-2-[1′-Hydroxy-2′-propenyl]-5-[1″-hydroxytridecyl]-tetrahydrofuran (3)

In a 50 mL round-bottomed flask equipped with magnetic stirring bar were placed CuBr (36.3 mg, 0.253 mmol) and anhydrous THF (10 mL). Pre-prepared $C_{11}H_{23}MgBr$ (11.5 mL, ca. 0.4 M in THF) was added dropwise at 0 °C, and then epoxide **5**[10b] (120 mg, 0.422 mmol) was introduced. The reaction mixture was stirred at 0 °C for 3 h, then poured into ice-cold saturated aqueous NH_4Cl, and extracted with ether. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC (2–5% EtOAc:petroleum ether) to give the derived alcohol (160 mg, 86%): R_f = 0.45 (5% EtOAc: petroleum ether); ^1H NMR ($CDCl_3$) δ 5.81 (m, 1H), 5.24–5.28 (dd, 2H, *J* = 17.1 Hz, 10.6 Hz), 4.10 (t, 1H), 3.88–3.89 (q, 1H), 3.75–3.76 (q, 1H), 3.35 (m, 1H), 2.31 (d, 1H, *J* = 4.1 Hz), 1.86–1.89 (m, 2H), 1.52–1.70 (m, 2H), 1.36 (m, 1H), 1.36–1.38 (m, 2H), 1.23 (s, br, 19H), 0.88 (s, br, 9H), 0.85 (t, *J* = 6.7 Hz, 3H), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.6, 115.7, 82.7, 82.4, 76.0, 74.0, 33.6, 31.9, 29.7 (two signals), 29.6 (three signals), 29.3, 28.4, 27.8, 25.8, 25.6, 22.7, 18.3, 14.1, 1.0, −4.6, −4.8; HRMS (FAB) calcd for $C_{26}H_{53}O_3Si$ ($M+H^+$) 441.3764, found 441.3744.

To a solution of the product from the previous step (64 mg, 0.145 mmol) in THF (10 mL) was added Bu_4NF (1.45 mL, 1 M in THF) at 0 °C. The reaction mixture was stirred at rt until TLC indicated complete disappearence of the starting material, at which time the mixture was diluted with water and extracted with EtOAc. The organic phase was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give **3** (40 mg, 85%): white powder; R_f = 0.3 (25% EtOAc: petroleum ether); ^1H NMR ($CDCl_3$) δ 5.78 (m, 1H), 5.18–5.36 (dd, 2H, *J* = 17.5, 10.5 Hz), 3.93 (m, 1H), 3.80–3.88 (m, 2H), 3.39 (m, 1H), 2.45 (b, 1H), 2.25 (b, 1H), 1.94–1.98 (m, 2H), 1.68–1.73 (m, 2H), 1.40–1.41 (m, 1H), 1.29–1.38 (m, 3H), 1.24 (s, br, 18H), 0.86 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 136.7, 117.1, 82.9, 82.2, 75.5, 74.0, 33.6, 31.9, 29.7 (three signals), 29.6 (three signals), 29.3, 28.5, 28.4, 25.6, 22.7, 14.1; HRMS (FAB) calcd for $C_{20}H_{38}O_3Na$ ($M+Na^+$) 349.2719, found 349.2708.

4.1.2. Methyl 8-(oxiran-2-yl)octanoate (7)

To a solution of methyl dec-9-enoate (1.40 g, 7.60 mmol) in CH_2Cl_2 (25 mL) was added *m*CPBA (4.26 g, 19 mmol, 77% purity) at room temperature. The mixture was stirred for 1 h and quenched by adding saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$, and extracted with EtOAc. The organic phase

was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC to give **7** (1.31 g, 86.4%) as a yellow oil; R_f = 0.70 (10% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 3H), 2.92 (m, 1H), 2.75–2.77 (m, 1H), 2.47–2.48 (m, 1H), 2.32 (t, J = 7.5 Hz, 2H), 1.60–1.62 (m, 2H), 1.34–1.55 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 174.2, 52.3, 51.4, 47.1, 34.1, 32.4, 29.2, 29.1, 29.0, 25.9, 24.9.

4.1.3. (*R*)-Methyl 8-(oxiran-2-yl)octanoate [(*R*)-7]

A solution of (*R*/*S*)-**7** (1.83 g, 9.17 mmol) in DME (10 mL) was treated with (*R*,*R*)-salen-Co(OAc) (182 mg, 0.275 mmol) and distilled water (0.091 mL, 5.06 mmol). The mixture was stirred at room temperature for 72 h, then purified by FCC to give (*R*)-**7** (677 mg, 37%) and the derived diol (765 mg, 38%). The TLC and NMR data for (*R*)-**7** are as listed for (*R*/*S*)-**7**.

4.1.4. (*S*)-Methyl 8-(oxiran-2-yl)octanoate [(*S*)-7]

(*S*)-**7** (4.2 g, 46%) was obtained from (*R*/*S*)-**7** (9.2 g. 46 mmol) using (*S*,*S*)-**8**, following the procedure described for (*R*)-**7**. The TLC and NMR data for (*S*)-**7** are as listed for (*R*/*S*)-**7**.

4.1.5. (*S*)-methyl 9-tert-butyldiphenylsilyloxytetradec-13-enoate [(*S*)-9]

To a suspension of CuBr (258 mg, 1.8 mmol) in THF (15 mL) at 0 °C was added 3-butenylmagnesium bromide (18 mL, 0.5 M in THF, 9.0 mmol) for over 5 min. A solution of (*S*)-**7** (600 mg, 3.00 mmol) in anhydrous THF (3 mL) was then introduced, dropwise over 5 min. The reaction mixture was stirred at 0 °C for 10 min at which time saturated aqueous NH_4Cl was added and the mixture was extracted with ether. The organic phase was washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give the derived alcohol: R_f = 0.50 (20% EtOAc: petroleum ether).

A solution of the product from the previous step (358 mg, 1.4 mmol), TBDPSCl (769 mg, 2.8 mmol), and imidazole (477 mg, 7.00 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred under nitrogen at room temperature for 2 h. The mixture was quenched with brine and extracted with ether (3 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to afford **9** (600 mg, 87%) as a pale yellow oil. R_f = 0.50 (5% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ *7.69–7.70* (m, 4H), 7.37–7.44 (m, 6H), 5.71–5.74 (m, 1H), 4.91–4.96 (m, 2H), 3.75 (m, 1H), 3.70 (s, 3H), 2.30 (t, J = 7.5 Hz, 2H), 1.92–1.93 (m, 2H), 1.59–1.60 (m, 2H), 1.40–1.45 (m, 6H), 1.14–1.23 (m, 8H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.5, 139.2, 136.2, 135.1, 135.0, 129.6, 127.6, 114.5, 77.0, 73.3, 51.6, 36.5, 36.0, 34.3, 34.0, 29.7, 29.3 (two signals), 27.3, 25.1, 25.0, 24.4, 19.6.

4.1.6. (*R*)-methyl 9-*tert*-butyldiphenylsilyloxytetradec-13-enoate [(*R*)-9]

(*R*)-**9** (600 mg, 75%) was obtained from (*R*)-**7** (324 mg. 1.62 mmol) following the two-step reaction sequence described for (*S*)-**9**. The TLC and NMR data for (*R*)-**9** was as listed for (*S*)-**9**.

4.1.7. Butenolide (*R*)-11

To a solution of diisopropylamine (1.2 mL, 8.5 mmol) in anhydrous THF (15 mL) at −78 °C was added BuLi (2.4 mL, 2.5 M in hexane, 6 mmol). The mixture was warmed to 0 °C and stirred at this temperature for 10 min, then cooled to −78 °C, at which time a solution of (*R*)-**9** (600 mg, 1.21 mmol) in anhydrous THF (6 mL) was added. The reaction mixture was stirred at −78 °C for 1 h and a solution of aldehyde **10**[14] (574 mg, 3.63 mmol) in anhydrous THF (6 mL) was slowly introduced. After 30 min, the reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with ether. The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by FCC to afford the aldol product as clear syrup (400 mg, 70%) based on consumed (*R*)-**9**.

To the solution of product from the previous step (400 mg, 0.613 mmol) in a mixture of MeOH (10 mL) and 2-propanol (1 mL) was added TsOH (12 mg, 0.06 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 20 h, then concentrated under reduced pressure. The residue was purified by FCC to afford the derived lactone as a yellow oil (227 mg, 69%).

To a solution of the material from the previous step (227 mg, 0.42 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (0.3 mL, 2.1 mmol) and MsCl (0.07 mL, 0.84 mmol) at 0 °C. After stirring at room temperature for 14 h, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with ether. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by FCC to afford (*R*)-**11** (176 mg, 81%) as a clear oil. R_f = 0.75 (15% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ *7.66–7.68* (m, 4H), 7.35–7.41 (m, 6H), 6.94 (d, J = 1.5 Hz, 1H), 5.69–5.72 (m, 1H), 4.97–4.99 (m, 1H), 4.88–4.94 (m, 2H), 3.70–3.74 (m, 1H), 2.20–2.24 (m, 2H), 1.90–1.91 (m, 2H), 1.38–1.47 (m, 11H), 1.14–1.1.23 (m, 6H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.8, 148.8, 138.9, 135.9, 134.8, 134.7, 134.3, 129.4 (two signals), 127.4, 114.3, 73.1, 36.3, 35.8, 33.8, 29.3, 29.1, 27.3, 27.1, 25.1, 24.7, 24.2, 19.4, 19.2; HRMS (FAB) calcd for $C_{33}H_{47}O_3Si$ ($M+H^+$) 519.3294, found 519.3282.

4.1.8. Butenolide (*S*)-11

Colorless oil; R_f = 0.70 (15% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ *7.69–7.70* (m, 4H), 7.37–7.45 (m, 6H), 6.96 (d, J = 1.4 Hz, 1H), 5.71–5.76 (m, 1H), 5.00–5.01 (m, 1H), 4.91–4.96 (m, 2H), 3.74–3.76 (m, 1H), 2.23–2.26 (t, J = 8.1 Hz, 2H), 1.92–1.93 (m, 2H), 1.25–1.49 (m, 11H), 1.16–1.23 (m, 6H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.8, 148.7, 138.9, 135.9, 134.8, 134.7, 134.4, 129.4, 127.4, 114.3, 73.1, 36.3, 35.8, 33.8, 29.3, 29.1, 27.3, 27.1, 25.1, 24.7, 24.2, 19.4, 19.2; HRMS (FAB) calcd for $C_{33}H_{47}O_3Si$ ($M+H^+$) 519.3294, found 519.3293.

4.1.9. Butenolide (*S*)-4

AcCl (5%) in MeOH (2.1 mL) was added at room temperature to a solution of (*S*)-**11** in CH_2Cl_2 (5 mL). The mixture was stirred for 16 h, then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. FCC of the residue afforded (*S*)-**4** (50 mg, 82%): clear oil; R_f = 0.45 (30% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.00 s, 1H), 5.81–5.86 (m, 1H), 4.97–5.06 (m, 3H), 3.61–3.62 (m, 1H), 2.27–2.31 (m, 2H), 2.02–2.11 (m, 2H), 1.37–1.58 (m, 18H); ^{13}C NMR ($CDCl_3$) δ 173.8, 148.8, 138.7, 134.3, 114.6, 71.8, 37.4, 36.9, 33.7, 29.3, 29.1, 27.4, 25.5, 24.9, 19.2; HRMS (FAB) calcd for $C_{17}H_{29}O_3$ ($M+H^+$) 281.2117, found 281.2111.

4.1.10. THF-butenolide (*R*)-12

Grubb's II catalyst (6 mg, 0.007 mmol) in CH_2Cl_2 (1 mL) was injected, at rt, into a degassed solution of alcohol **3** (23 mg, 0.07 mmol) and (*R*)-**11** (74 mg, 0.14 mmol), in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 20 h at rt, then quenched by addition of DMSO (50 μL) and concentrated in vacuo. FCC of the residue afforded (*R*)-**12** (42 mg, 73% based on **3**), recovered (*R*)-**11** (ca. 15%), and the homodimer (*R*)-**11** (ca. 20%). For (*R*)-**12**: clear oil; R_f = 0.50 (30% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ *7.65–7.67* (m, 4H), 7.34–7.42 (m, 6H), 6.94 (s, 1H), 5.65–5.68 (m, 1H), 5.32–5.34 (m, 1H), 4.97–4.99 (m, 1H), 3.81–3.85 (m, 3H), 3.70–3.73 (m, 1H), 3.41 (m, 1H), 2.43–2.44 (m, 1H), 2.20–2.21 (m, 3H), 1.88–1.89 (m, 4H), 1.20–1.47 (m, 41H), 1.04 (s, 9H), 0.89 (t, J = 3.2 Hz, 3H); ^{13}C NMR (CDCl3) δ 173.8, 149.5, 149.3, 149.1, 148.7, 135.9, 134.7, 134.5, 134.3, 129.4, 128.3, 127.4, 123.1, 123.0 122.7, 82.7, 82.6, 75.6, 74.0, 73.0, 33.6, 31.9, 29.7 (three signals), 29,6 (three signals), 29.3 (two signals), 29.1, 28.4 (two signals),

27.3, 27.1, 25.1, 22.7, 19.2, 14.1; HRMS calcd for $C_{51}H_{80}O_6SiNa$ ($M+Na^+$) 839.5622, found, 839.5613.

4.1.11. THF-butenolide (*S*)-13

Grubb's II catalyst (9.3 mg, 0.01 mmol) in CH_2Cl_2 (1 mL) was injected, at rt, into a degassed solution of alcohol **3** (36 mg, 0.11 mmol), and (*S*)-**4** (50 mg, 0.178 mmol), in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 20 h at rt, then quenched by addition of DMSO (50 μL) and concentrated in vacuo. FCC of the residue provided (*S*)-**13** (36 mg, 57% based on **3**), and the homodimer of (*S*)-**4** (ca. 27%). For (*S*)-**13**: clear oil; R_f = 0.50 (80% EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ 7.01 (s, 1H), 5.76–5.82 (m, 1H), 5.41–5.46 (m, 1H), 5.02–5.03 (m, 1H), 3.85–3.92 (m, 3H), 3.61 (s, br, 1H), 3.42–3.44 (m, 1H), 2.29 (t, J = 7.5 Hz, 2H), 2.10 (m, 2H), 1.97–2.01 (m, 2H), 1.28–1.70 (m, 44H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.9, 148.9, 134.3, 128.6, 82.8, 82.5, 76.8, 76.7, 75.5, 74.0, 71.7, 37.4, 36.9, 32.3, 31.9, 29.7 (five signals), 29.6, 29.4, 29.3, 29.2, 27.4, 25.6, 25.5, 25.2, 22.7, 19.2, 14.2; HRMS (FAB) calcd for $C_{35}H_{63}O_6$ ($M+H^+$) 579.4614, found 579.4625.

4.1.12. (9*R*)-4-deoxyannoreticuin (1)

Chlorotris(triphenylphosphine)-rhodium (**I**) (7.1 mg, 0.0077 mmol) was added to a degassed solution of (*R*)-**12** (23 mg, 0.028 μmol) in a mixture of benzene–EtOH (2 mL, 50% v/v). The mixture was stirred under an atmosphere of hydrogen for 12 h, at which time the solvent was removed under reduced pressure. FCC of the residue gave an inseparable mixture of the 13,14-dihydro- and tetrahydro derivatives of (*R*)-**12** (16 mg, 70%): R_f = 0.50 (30% EtOAc: petroleum ether).

A mixture of 5% AcCl in MeOH (0.5 mL) was added at room temperature to a solution of the material obtained in the previous step (10 mg, 12.2 μmol) in CH_2Cl_2 (1 mL). The mixture was stirred at this temperature for 3 h, diluted with CH_2Cl_2, and washed with a saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. FCC of the residue afforded a mixture of **1** and the completely hydrogenated product (7.2 mg, 72%) in an approximate 7:1 ratio: R_f = 0.35 (60% EtOAc: petroleum ether). Repeated FCC provided a sample of **1** that contained less than 10% of the completely reduced material as a white waxy solid; $[\alpha]_D^{22}$ +19 (*c* 2.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1H), 4.96–4.98 (m, 1H), 3.76–3.80 (m, 2H), 3.56 (s, br, 1H), 3.36–3.41 (m, 2H), 2.25–2.27 (m, 4H, CH_2, 2× OH, D_2O ex.), 1.96–1.97 (m, 2H), 1.24–1.52 (m, 48H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.8, 148.9, 134.3, 82.7, 77.4, 74.0 (two signals), 71.9, 37.4, 33.5, 33.4, 31.9, 29.7 (three signals), 29.6 (three signals), 29.3, 29.1, 28.7, 27.3, 25.6 (three signals), 25.5, 25.2, 22.7, 19.2, 14.1; HRMS (FAB) calcd for $C_{35}H_{65}O_6$ (M+H) 581.4781, found 581.4794.

4.1.13. (9*S*)-4-deoxyannoreticuin (2)

A solution of sodium acetate (280 mg, 3.41 mmol) in water (5 mL) was added via a syringe pump, for over 4 h, to a mixture of (*S*)-**13** (24 mg, 0.041 mmol), *p*-toluenesulfonyl hydrazide (511 mg, 2.74 mmol), and DME (4 mL) at reflux. After cooling to rt, the reaction mixture was poured into water and extracted with EtOAc. The combined organic extract was washed with 2 M HCl, water, and brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and purified by FCC to give **2** as a white waxy solid (21.5 mg, 90%). R_f = 0.50 (80% EtOAc: petroleum ether); $[\alpha]_D^{22}$ +25 (c 4.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 1H), 4.95–4.99 (m, 1H), 3.76–3.80 (m, 2H), 3.56 (s, br, 1H), 3.36–3.40 (m, 2H), 2.23–2.24 (m, 2H), 1.95–1.96 (m, 2H), 1.66 (m, 2H), 1.23–1.53 (m, 48H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.8, 148.9, 134.3, 82.7, 82.6, 77.4, 74.0, 71.9, 37.4, 33.5, 33.4, 31.9, 29.7, 29.6, 29.3, 29.1, 28.7, 27.4, 25.6, 25.5, (two signals), 22.7, 19.2, 14.1; HRMS calcd for $C_{35}H_{65}O_6$ (M+H) 581.4781, found 581.4789.

4.2. Cytotoxicity assay

Cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and penicillin-streptomycin-fungizone mixture (100 U/mL, 100 μg/mL, and 0.25 μg mL, respectively) and maintained in a 37 °C humidified 5% CO_2 incubator. On the day before the drug treatment, cells were plated onto each well of 96-well plate at 2000 cells/well (200 μl of the medium per well). After 24 h, cells were treated with different concentrations of compounds and incubated for 72 h. After the incubation, cell growth was evaluated using a CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). UV absorption (490 nm) of each well was quantified by SpectraMax Plus 384 microplate reader (Molecular Devices). The data are provided as Supplementary Information.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.08.030.

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