From Natural Taxuspine X to structurally simplified Taxane Analogues: Synthesis of "Non-Natural" Natural Products with Remarkable P-gp Modulating Activity

Daniele Castagnolo,[a] Lorenzo Contemori,[a] Giorgio Maccari,[a] Stanislava I. Avramova,[a] Annalisa Neri,[c,d] Gianpietro Sgaragli,[c,d] Maurizio Botta [*,a,b]

[a] *Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Alcide de Gasperi 2, 53100 Siena, Italy.* [b]*Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple UniVersity, BioLife Science Building, Suite 333, 1900 N 12th Street, Philadelphia, Pennsylvania 19122, USA.* [c]*Dipartimento di Neuroscienze, Università degli Studi di Siena, via A. Moro 2, 53100 Siena, Italy.* [d]*Istituto Toscano Tumori, via T. Alderotti 26N, 50139 Firenze, Italy.* E-mail: botta.maurizio@gmail.com

Supporting Information

Table of Contents

General

All commercially available chemicals were used as purchased. CH_2Cl_2 was dried over sodium hydride. THF and Toluene were dried over Na/benzophenone prior to use. Anhydrous reactions were run under a positive pressure of dry N_2 or argon.

Instrumentation

^{1}H-NMR and ^{13}C-NMR spectra were measured on a 200 MHz and on a 400 MHz spectrometers. Chemical Shifts for protons are reported in parts per million (δ scale) and internally referenced to the $CDCl_3$ signal at δ 7.24 ppm. Chemical Shifts for carbon are reported in parts per million n (δ scale) and referenced to the carbon resonances of the solvent (CDCl3: δ 77.76 ppm, the middle peak). Data is presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiplet resonances, br = broad), coupling constant in Hertz (Hz), and integration. IR spectra were recorded on a Perkin-Elmer BX FTIR system, using KBr pellets. Mass spectra (MS) data were obtained using a LC/MSD VL system with a 0.4 mL/min flow rate using a binary solvent system of 95:5 methyl alcohol/ water. UV detection was monitored at 254 nm. Mass spectra were acquired in positive and negative mode scanning over the mass range. Elemental analyses (C, H, N) were performed in house.

HPLC and MS analysis

The purity of compounds was assessed by reverse-phase liquid chromatography and a mass spectrometer with a UV detector at λ = 254 nm and an electrospray ionization source (ESI). All the solvents were HPLC grade. Mass spectral (MS) data were obtained using a LC/MSD VL system with a 0.4 mL/min flow rate using a binary solvent system of 95:5 methyl alcohol/water. UV detection was monitored at 254 nm. Mass spectra were acquired in positive mode scanning over the mass range of 50-1500. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulize pressure, 40 psig; drying gas temperature, 350 °C.

Computational Methods

Ligand structures Preparation: All the ligands structures are energy minimized with MacroModel[1] software using OPLS-2005[2] forcefield ad conjugated gradient minimizer, stop conditions was fixed to a gradient convergence of 0.01 Kcal/Å² or a maximum of 10000 iterations.

Pharmacophore Generation: The pharmacophore preparation was done using the "Common Feature Pharmacophore Generation" protocol of the Discovery Studio suite,[3] setting a total number of features to 5 with an inter-feature distance of 2.0 Å, a maximum of 500 conformations for each ligands are generated using the BEST algorithm whit an energy threshold of 20.0 Kcal/mol; Among the 100 best pharmacophores generated was choose the best in fit value. To generate the hypothesis was used the four compounds retrieved in literature.[4]

Ligand Alignment: The three compounds presented in this work are aligned to the pharmacophore using the "Ligand Pharmacophore Mapping" of Discovery Studio. Conformations generation parameters are the same used for generate the hypothesis, all the features must be mapped.

Pharmacological evaluation

In order to assay the P-gp function we have followed the method already described elsewhere[5] and here summarized.

Chemicals

McCoy's 5A medium, heat-inactivated horse serum, L-glutamine, sodium orthovanadate (Vi), colchicine, rhodamine 123 were purchased from Sigma Chemical Co. (Milan, Italy), penicillin (10,000 UI/ml) and streptomicin (10 mg/ml) mixture from Lonza (Basle, Switzerland).

Cells line and cultures

The L5178Y mouse T-lymphoma parent cell line transfected with a recombinant MDR1/A retroviral vector (pHa MDR1/A)[6] was a generous gift from Dr. Michael M. Gottesman (National Cancer Institute, Bethesda, MD, USA). MDR1-expressing cells were selected by culturing the transfected cells with 60 ng ml^{-1} colchicine to maintain the expression of the MDR phenotype.[7] The L5178 MDR1 cell line was grown in McCoy's 5A medium supplemented with 10% heat-inactivated horse serum, 2 mM L-glutamine, 100 UI/ml penicillin and 0.01 mg/ml streptomycin. Cells were maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37°C. When the cells reached confluency, they were harvested and plated for subsequent passages (up to 20) and for drug treatment. Cultures initiated at a density of $2x10^5$ cells/ml and grew exponentially to about $2x10^6$ cells/ml in 48h. Cultured cells were counted with use of a Burker cytometer before use. The viability of cells, tested by Trypan Blue exclusion, was always greater than 95%.

Cell loading with R123 and inhibition of Pgp-mediated R123 efflux assay

The ability of the synthesized compounds to inhibit R123 efflux was determined in the following way. Briefly, L5178 MDR1 cells ($2x10^6$ ml^{-1}) were resuspended in serum-free McCoy's 5A medium, and 0.5 ml aliquots of the cell suspension were distributed into Eppendorf centrifuge tubes. Compounds to be tested were added at different concentrations and samples were incubated for 10 min at room temperature. Then, R123 indicator was added to the samples at a final concentration of 5.2 µM and cells were incubated for 20 min at 37°C. Thereafter, cells were washed twice by centrifugation for 5 min at 2,000 *g* and resuspended in 0.5 ml phosphate-buffered saline (PBS). The incubation period of 20 min, used to expose cells to R123 in our assay system, was sufficient for cells to attain a steady-state concentration of R123 even in the presence of V_i; in this condition, in fact, the fluorescence values obtained were much higher and already maximal at about 5 µM R123 concentration which was selected for use in the assay (data not shown). R123 retained by cells was quantified as fluorescence, using a Becton-Dickinson FACS Calibur flow cytometer (San Josè, CA, USA) equipped with an ultraviolet argon laser (excitation at 488 nm, emission at 530/30 and 585/42 nm band-pass filters). FACS analysis was gated to include only individual, viable cells on the basis of forward and side light-scatter and was based on acquisition of data from 10,000 cells. Fluorescence signals were analyzed by the BDIS CellQuest software (Becton Dickinson, San Josè, CA, USA). The mean fluorescence intensity (MFI) was used for comparison among different conditions. V_i was selected as a positive control for a standard inhibitor because already at 5 mM concentration it can maximally inactivate the Pgp efflux pump.[7]

Data analysis and statistics

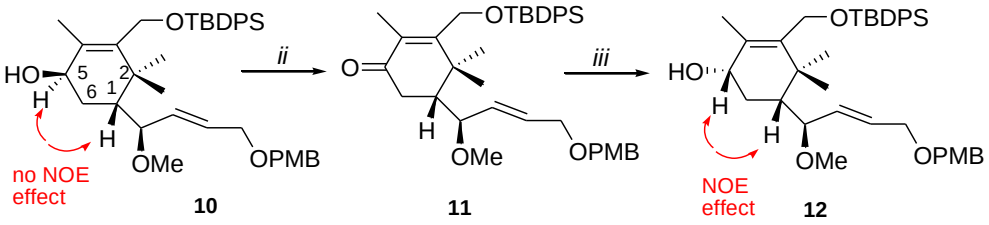
The fluorescence data are expressed as the mean of arbitrary fluorescence units derived from histogram plots of the 10,000 cells that were examined. All assays were performed at least in triplicate. The percent Pgp inhibition exerted by a single compound was calculated as described by Wang et al., (2000). The relative fluorescence (percent inhibition of Pgp) was calculated as mean fluorescence of a discrete sample divided by the mean fluorescence in the presence of 5 mM V_i, times 100 or expressed as:

Relative fluorescence = (MFI of sample/ MFI of sample + V_i) x 100

The denominator represents MFI of the sample when inactivation or complete preclusion of the function of Pgp active efflux is attained. The numerator is the resulting signal caused by test compound inhibiting the function of Pgp active efflux. Pgp blocking activity was described by α_{max},

which expresses the efficacy and by IC_{50}, which measures the potency of the inhibitor. α_{max} varies between 0 (in the absence of the inhibitor) and 1 (when the amount of R123 found in L5178 MDR1 cells was comparable to that determined in presence of 5 mM V_i). IC_{50} measures the potency of the inhibitor and represents the concentration that causes a half-maximal increase (α=0.5) in intracellular concentration of R123. IC_{50} values were obtained by best fitting the concentration-dependent inhibition data according to one-site or two-site models, by using GraphPadPrism (GraphPad Inc., USA). Data are the mean ±s.e. from triplicate samples of at least three independent experiments.

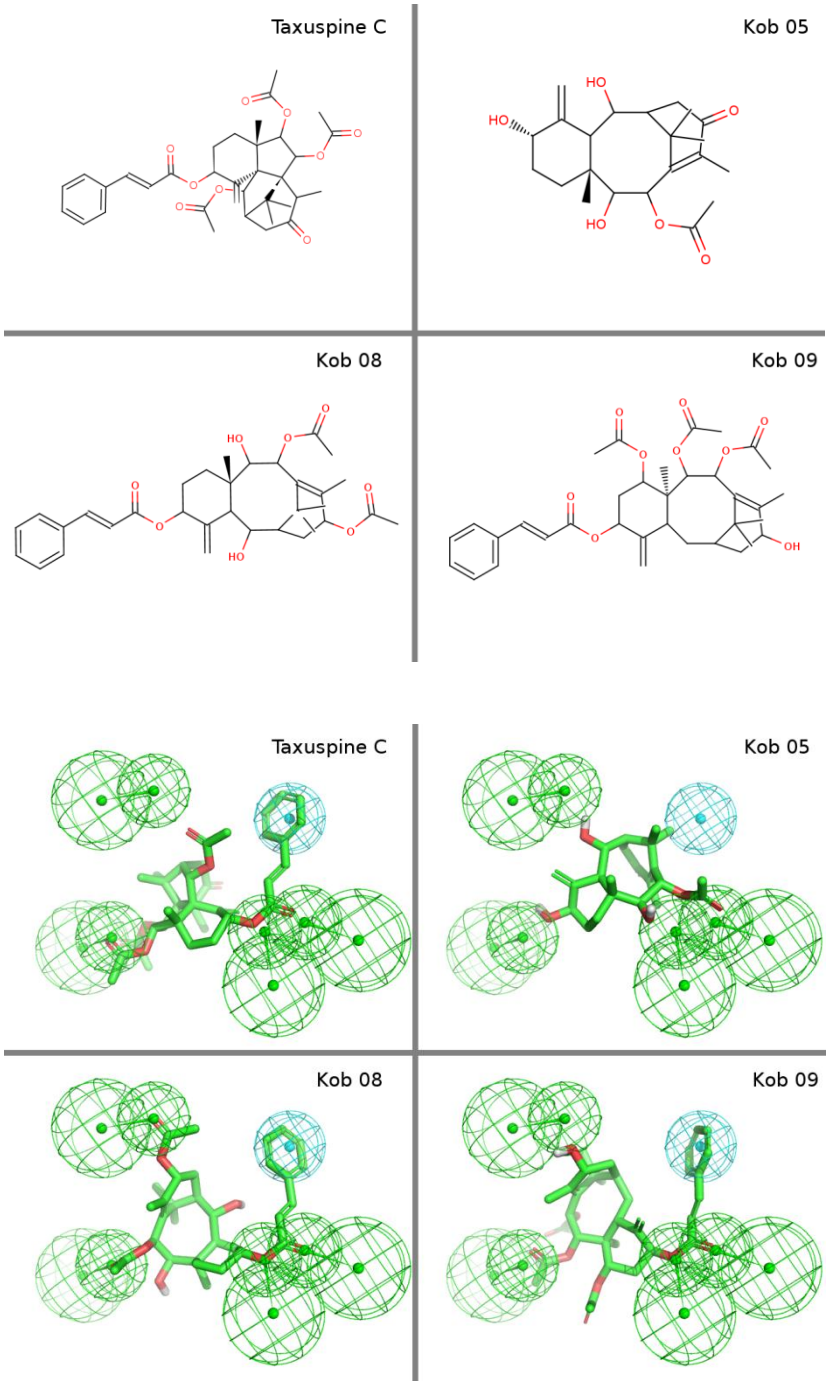
Determination of the relative stereochemistry of 10



Reagent and conditions: *i.* NMO, TPAP, mol. sieves, DCM, rt, 1h, (93%). *ii.* $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, rt, 10 min, (quant.).

Generation of the pharmacophoric model

Few examples of extensive studies on taxoids and taxuspine analogues test for the MDR reversal activity were reported till now.[8-9-10] Starting from Kobayashi's work published in 1998, we chose four taxanes endowed with a P-gp inhibitory activity comparable or higher than that of Verapamil. Structures of the four reference compounds were energy minimized and used to generate a qualitative pharmacophoric model. No ionization or tautomerization are required. The pharmacophoric hypothesis were generated with the "Common Feature Pharmacophore Generation" protocol of Discovery Studio and consisted of four hydrogen bond acceptor features and one hydrophobic feature; the latter one is well mapped by compounds bearing an aromatic ring.



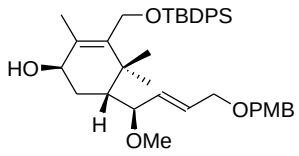
Generation of pharmacophoric model

Experimental procedures

Synthesis of aldehyde 8.

Alkene **9** was prepared according to previously reported procedures.[11]

Synthesis of alcohol 10

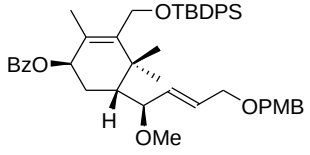


10

To a solution of **9** (64 mg, 0.1 mmol) in dry dioxane (3 mL), pyridine (16 µL, 0.2 mmol) and SeO_2 (22 mg, 0.2mmol) were added and the reaction mixture was heated to reflux for 1h. After cooling to rt, the mixture was diluted with Et_2O (20 mL) and quenched with a saturated solution of $NaHCO_3$ (10 mL). Organic phase was separated and washed with brine. The organic phase was dried over anhydrous Na_2SO_4, concentrated and purified by flash chromatography (Et_2O/petroleum ether 1:1) to afford compound **10** as colorless oil in 30 % yield.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.62 (d, *J* = 8.0 Hz, 4H), 7.32 (m, 6H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 5.64 (m, 2H), 4.39 (s, 2H), 4.06 (q, *J* = 15.6, 4.4 Hz, 2H), 3.96 (d, *J* = 4.4 Hz, 2H), 3.86 (bs, 1H), 3.74 (s, 4H), 3.13 (s, 3H), 1.71 (m, 2H), 1.63 (m, 3H), 1.51 (m, 1H), 1.00 (s, 12H), 0.81 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 159.2, 140.9, 135.9, 133.7, 133.1, 132.5, 130.3, 129.6, 129.4, 128.6, 128.0, 127.9, 127.8, 127.5, 113.9, 113.8, 80.3, 71.7, 69.9, 69.4, 65.0, 60.1, 56.1, 55.3, 44.2, 38.2, 27.2, 26.9, 26.3, 21.1, 19.3, 17.9 ppm. MS (ESI): *m/z* 629 $[M+H]^+$, 651 $[M+Na]^+$, 667 $[M+K]^+$. Anal. ($C_{39}H_{52}O_5Si$) C, H, N.

Synthesis of benzoyl derivative 13

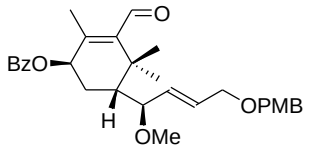


13

To a solution of **10** (100 mg, 0.16 mmol) in dry CH_2Cl_2 (10 mL), pyridine (104 µL, 1.28 mmol), benzoyl chloride (72 µL, 0.64 mmol), DMAP (5 mol%) and Bu_4NI (5 mol%) were added and the reaction mixture was stirred at rt for 12h. The mixture was diluted with Et_2O (20 mL) and then quenched with a HCl 1N solution (10 mL). Organic phase was separated. Aqueous layer was added with a saturated solution of $NaHCO_3$ (10 mL) until pH = 7, and then extracted twice with Et_2O (20

mL). Organic phases were collected, washed with brine (20 mL), dried over anhydrous Na_2SO_4, concentrated under vacuum to afford crude compound **13**. The crude was purified by flash chromatography (Et_2O/petroleum ether 1:5) to afford compound **13** as a colorless oil in 86 % yield. ^{1}H-NMR (200MHz, $CDCl_3$) δ: 8.16 (d, *J* = 8.7 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 1H), 7.77 (m, 3H), 7.42 (m, 10H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.85 (d, *J* = 8.3 Hz, 2H), 5.72 (m, 2H), 5.58 (bs, 1H), 4.32 (m, 4H), 3.97 (d, *J* = 4.5 Hz, 2H), 3.91 (d, *J* = 4.5 Hz, 1H), 3.78 (s, 3H), 3.25 (s, 3H), 2.06 (m, 2H), 1.83 (m, 1H), 1.70 (s, 3H), 1.26 (s, 3H), 1.17 (s, 9H), 1.05 (s, 3H) ppm. ^{13}C-NMR (50MHz, $CDCl_3$) δ: 171.1, 166.3, 158.9, 143.4, 135.6, 133.4, 133.3, 132.8, 132.5, 130.7, 129.9, 129.5, 129.5, 129.3, 129.2, 129.1, 128.2, 128.1, 127.9, 127.4, 127.4, 79.8, 72.6, 71.1, 69.3, 65.6, 59.9, 55.9, 54.9, 45.2, 38.0, 26.6, 26.4, 24.6, 21.0, 19.2, 17.5, 15.0 ppm. MS (ESI): *m/z* 755 $[M+Na]^+$. Anal. ($C_{46}H_{56}O_6Si$) C, H, N.

Synthesis of aldehyde 14



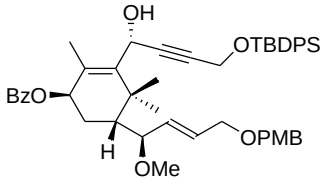
14

A solution of silyl ether **13** (100 mg, 0.14 mmol) in dry THF (5 mL) was treated with TBAF (1M solution, 680 µL, 0.68 mmol) and the resulting solution was stirred at 40 °C for 14h. The reaction was then allowed to reach rt and concentrated under vacuum. Crude compound was filtered through a pad of silica gel (hexanes/Et_2O 1:3) affording the corresponding primary alcohol as colorless oil in 90% yield. Primary alcohol was directly used in the next step. Tetra(*n*-propyl)ammonium perruthenate (10 mol%) was added portionwise to a suspension of 100 mg (0.2 mmol) of that alcohol intermediate in dry CH_2Cl_2 (4 mL), molecular sieves 4Å (50 mg) and 4-methylmorpholine-N-oxide (90 mg, 0.8 mmol) at 0°C. The reaction mixture was stirred for 2h at rt and then filtered through a Celite pad. The filtrate was concentrated in vacuum and purified by flash chromatography (hexanes/Et_2O 1:2) to afford the aldehyde **14** as colorless oil in quantitative yield.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 10.10 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.72 (d, *J* = 8.0 Hz, 2H), 5.66 (m, 1H), 5.54 (m, 2H), 4.18 (q, *J* = 16.0 Hz, *J* = 8.0 Hz, 2H), 3.82 (m, 3H), 3.68 (s, 3H), 3.11 (s, 3H), 2.01 (s, 3H), 1.90 (m, 2H), 1.61 (m, 1H), 1.29 (s, 3H), 1.11 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 193.6, 166.0, 159.1, 147.0, 143.9, 133.0, 132.2, 120.2, 130.1, 129.6, 129.3, 128.5, 128.4, 113.7, 79.2, 72.4, 71.4,

69.5, 55.1, 55.2, 45.9, 37.1, 26.7, 24.3, 20.7, 16.7 ppm. MS (ESI): *m/z* 515 $[M+Na]^+$. Anal. ($C_{30}H_{36}O_6$) C, H, N.

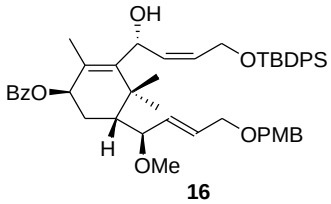
Synthesis of propargyl alcohol 15



15

A solution of O-TBDPS-propargyl alcohol (180 mg, 0.6 mmol)[12] in THF (5 mL) was cooled to -78 °C and treated with 2.5M solution of *n*BuLi (200 µL, 0.5 mmol). The mixture was stirred at -78 °C for 30 min. A solution of the aldehyde **14** (100 mg, 0.2 mmol) in THF (5 mL) was then added to the first solution and the resulting reaction mixture was stirred at -20 °C for 2h. The reaction was then left to reach rt overnight. The mixture was diluted with Et_2O (15 mL) and aqueous NH_4Cl (20 mL) was added. The organic phase was separated and then dried (Na_2SO_4), concentrated and purified by flash chromatography (Et_2O/hexanes 1:1) to afford compound **15** as colorless oil in 90% yield.
^{1}H-NMR (200MHz, $CDCl_3$) δ: 8.00 (d, *J* = 7.2 Hz, 2H), 7.74 (m, 4H), 7.39 (m, 9H), 7.12 (d, *J* = 8.61 Hz, 2H), 6.81 (d, *J* = 8.46 Hz, 2H), 5.72 (m, 2H), 5.47 (s, 1H), 5.10 (s, 1H), 4.41 (s, 2H), 4.25 (d, *J* = 2.1 Hz, 2H), 3.90 (d, *J* = 5 Hz, 2H), 3.84 (d, *J* = 6 Hz, 1H), 3.77 (s, 3H), 3.22 (s, 3H), 2.30 (bs, 1H), 1.95 (m, 5H), 1.78 (m, 1H), 1.27 (s, 3H), 1.10 (s, 9H), 1.01 (s, 3H) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 166.5, 159.1, 144.2, 135.7, 135.5, 133.2, 132.7, 131.1, 130.6, 130.2, 129.9, 129.8, 129.6, 129.4, 128.3, 127.7, 113.7, 85.9, 83.0, 80.7, 80.3, 79.9, 79.7, 73.1, 71.4, 69.6, 59.6, 52.9, 45.5, 44.9, 38.8, 26.7, 20.7, 19.2 ppm. MS (ESI): *m/z* 809 $[M+Na]^+$. Anal. ($C_{49}H_{58}O_7Si$) C, H, N.

Synthesis of alkene 16

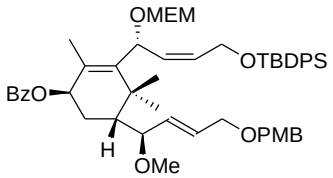


16

To a solution of alkyne **15** (80 mg, 0.1 mmol) in ethyl acetate (10 mL) was added quinoline (12 µL, 0.1 mmol) and Lindlar's catalyst (80 mg). The suspension was stirred under hydrogen atmosphere for 2h and then filtered through a Celite pad. The filtrate was concentrated and purified by flash chromatography (Et_2O/petroleum ether 1:1) to give *Z*-olefine **16** as a colorless oil in 80% yield.

^{1}H-NMR (200MHz, $CDCl_3$) δ: 8.00 (d, *J* = 7.2 Hz, 2H), 7.73 (m, 4H), 7.41 (m, 9H), 7.12 (d, *J* = 8.57 Hz, 2H), 6.80 (d, *J* = 8.63 Hz, 2H), 5.82 (m, 1H), 5.67 (m, 3H), 5.44 (s, 1H), 5.13 (bs, 1H), 4.42 (d, *J* = 5.26 Hz, 2H), 4.24 (d, *J* = 1.09 Hz, 2H), 3.88 (d, *J* = 5 Hz, 2H), 3.82 (d, *J* = 6.1 Hz, 1H), 3.78 (s, 3H), 3.19 (s, 3H), 2.76 (d, *J* = 2.5 Hz, 1H), 1.86 (m, 5H), 1.74 (m, 1H), 1.27 (s, 3H), 1.09 (s, 9H), 0.89 (s, 3H) ppm. ^{13}C-NMR (50MHz, $CDCl_3$) δ: 166.4, 159.0, 146.2, 135.4, 135.4, 134.5, 133.0, 132.9, 132.6, 132.5, 130.6, 130.1, 130.1, 129.7, 129.6, 129.5, 129.2, 128.1, 127.7, 113.6, 79.9, 73.1, 71.2, 69.5, 66.4, 60.9, 56.0, 55.1, 45.2, 36.6, 26.7, 26.5, 20.5, 19.0, 18.5 ppm. MS (ESI): *m/z* 811 $[M+Na]^+$. Anal. ($C_{49}H_{60}O_7Si$) C, H, N.

Synthesis of alkene 17

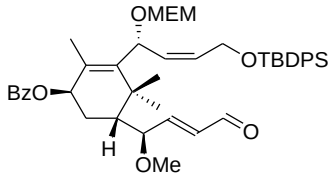


17

To a solution of alkene **16** (140 mg, 0.18 mmol) in dry CH_2Cl_2 (6 mL), DIPEA (280 µL, 2.16 mmol) was added followed by MEMCl (122 µl, 1 mmol), Bu_4NI (5 mol%) and DMAP (5 mol%). The reaction mixture was stirred at 40 °C for 2h. After cooling to rt, the organic phase was diluted with CH_2Cl_2, washed with water twice (10 mL), concentrated and dried over Na_2SO_4. The crude mixture was purified by flash chromatography (Et_2O/petroleum ether 1:1) to afford compound **17** as colorless oil in 83% yield.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.93 (d, *J* = 7.0 Hz, 2H), 7.69 (m, 4H), 7.51 (m, 9H), 7.09 (d, *J* = 8.52 Hz, 2H), 6.77 (d, *J* = 8.73 Hz, 2H), 5.81 (m, 1H), 5.69 (m, 2H), 5.61 (m, 1H), 5.39 (s, 1H), 4.96 (d, *J* = 8.7 Hz, 1H), 4.57 (s, 2H), 4.49 (m, 2H), 4.22 (s, 2H), 3.85 (d, *J* = 4.9 Hz, 2H), 3.80 (d, *J* = 6.1 Hz, 1H), 3.74 (s, 3H), 3.55 (m, 1H), 3.46 (m, 1H), 3.34 (m, 2H), 3.22 (s, 3H), 3.17 (s, 3H), 1.83 (m, 5H), 1.71 (m, 1H), 1.29 (s, 3H), 1.06 (s, 9H), 0.86 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 166.2, 143.8, 135.6, 133.6, 132.6, 132.5, 130.9, 130.5, 130.2, 129.7, 129.4, 129.2, 128.8, 128.4, 128.2, 127.7, 113.5, 91.7, 80.1, 73.6, 71.7, 71.3, 69.7, 69.1, 67.0, 60.2, 58.6, 55.9, 55.1, 45.6, 38.8, 26.8, 26.6, 24.5, 21.1, 19.0, 18.5 ppm. MS (ESI): *m/z* 900 $[M+Na]^+$. Anal. ($C_{53}H_{68}O_9Si$) C, H, N.

Synthesis of aldehyde 19

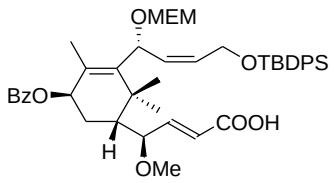


19

A solution of p-methoxybenzyl ether **17** (200 mg, 0.228 mmol) in CH_2Cl_2/phosphate buffer (8 mL : 0.8 mL) was treated with DDQ (52 mg, 0.228 mmol) and the resulting solution was stirred at rt for 30 min. After this period the reaction was filtered through a Celite pad and concentrated in vacuo. The residue was purified by flash chromatography (Et_2O/hexanes 3:1) to give the corresponding primary alcohol **18** (140 mg) as yellow oil in 97% yield. Primary alcohol was immediately used in the next step. Tetra(*n*-propyl)ammonium perruthenate (3.7 mg, 0.011 mmol) was added portionwise to a suspension of **18** (140 mg, 0.185 mmol) in CH_2Cl_2 (5 mL), molecular sieves 4Å (69 mg) and 4-methylmorpholine-N-oxide (30.8 mg, 0.263 mmol) at 0 °C. The reaction mixture was allowed to reach rt and then stirred for 1h. The mixture was then filtered through a Celite pad. The filtrate was concentrated and purified by flash chromatography (Et_2O/hexanes 3:1) to afford aldehyde **19** as colorless oil in quantitative yield. Compound **19** proved to be pure enough to be used in the next step without any further purification.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 9.37 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.66 (m, 4H), 7.37 (m, 9H), 6.60 (m, 1H), 6.14 (m, 1H), 5.79 (m, 1H), 5.62 (m, 1H), 5.36 (s, 1H), 4.94 (d, *J* = 8.0 Hz, 1H), 4.53 (s, 2H), 4.41 (m, 2H), 4.05 (d, *J* = 4.0 Hz, 1H), 3.52 (m, 1H), 3.42 (m, 1H), 3.32 (m, 2H), 3.20 (s, 3H), 3.18 (s, 3H), 1.77 (m, 6H), 1.21 (s, 3H), 1.01 (s, 9H), 0.82 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 192.8, 156.6, 143.3, 135.6, 133.7, 132.7, 132.1, 129.6, 129.3, 128.6, 128.3, 127.7, 91.7, 79.4, 73.1, 71.6, 69.0, 67.0, 60.2, 58.6, 56.9, 44.9, 38.8, 26.9, 26.5, 24.4, 21.0, 18.4 ppm. MS (ESI): *m/z* 777 $[M+Na]^+$. Anal. ($C_{45}H_{58}O_8Si$) C, H, N.

Synthesis of carboxylic acid 20



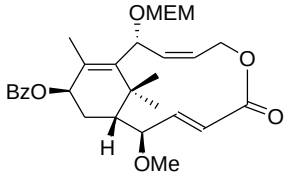
20

A mixture of $NaClO_2$ (96 mg, 1.06 mmol) and $NaH_2PO_4{\cdot}H_2O$ (218.7 mg, 1.58 mmol) in water (2 mL) was added to a solution of **19** (200 mg, 0.264 mmol) dissolved in 8 mL of ^{t}BuOH/2-methyl-2-butene (3:1). The resulting mixture was stirred at rt for 16h. The organic solvents were evaporated

and the crude product was purified by flash chromatography (EtOAc/hexane 1:1) to afford acid **20** as colorless oil in 84% yield.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.90 (d, *J* = 8.0 Hz, 2H), 7.67 (m, 4H), 7.42 (m, 9H), 6.84 (m, 1H), 5.92 (m, 1H), 5.81 (m, 1H), 5.65 (m, 1H), 5.39 (s, 1H), 4.95 (d, *J* = 8.0 Hz, 1H), 4.55 (s, 2H), 4.43 (m, 2H), 4.00 (d, *J* = 4.0 Hz, 1H), 3.55 (m, 1H), 3.45 (m, 1H), 3.32 (m, 2H), 3.22 (s, 3H), 3.20 (s, 3H), 1.79 (m, 6H), 1.22 (s, 3H), 1.04 (s, 9H), 0.83 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 170.7, 166.3, 150.5, 143.3, 135.6, 133.7, 132.7, 130.8, 130.5, 129.7, 129.5, 129.4, 128.7, 128.3, 127.7, 127.6, 120.5, 91.7, 79.2, 73.2, 71.7, 69.1, 67.0, 63.6, 60.2, 58.6, 56.9, 44.7, 38.8, 30.3, 26.9, 24.4, 21.0, 18.4 ppm. MS (ESI): *m/z* 793 $[M+Na]^+$. Anal. ($C_{45}H_{58}O_9Si$) C, H, N.

Synthesis of macrolactone 6



6

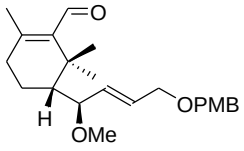
To a stirred solution of acid **20** (140 mg, 0.182 mmol) in dry THF (2 mL) was added TBAF (0.91 mmol) and the mixture was heated at 40 °C. After 2h the reaction mixture was cooled to rt, concentrated and then purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to give the *seco*-acid **21** as colorless oil in quantitative yield.

A solution of acid **21** (60mg, 0.113 mmol) in dry THF (3 mL) at 0 °C was treated with Et_3N (202 mmol) and 2,4,6-trichlorobenzoyl chloride (22.1 μL, 0.135 mmol). After 1h the mixture was diluted with THF (1mL) and toluene (1mL), and the resulting solution was added via syringe pump to a solution of DMAP in toluene (28 mL) at 75 °C over a period of 3h. After an additional 1h the solution was cooled at rt, diluted with AcOEt, washed with aq. NH_4Cl and then extracted with AcOEt (20 mL). The dried (over Na_2SO_4) extracts were concentrated under reduced pressure and purified by flash chromatography (Et_2O/hexanes 1:1) to afford lactone **6** in 50% yield as a colorless oil.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.96 (d, *J* = 8.0 Hz, 2H), 7.57 (t, *J* = 8.0 Hz, 1H), 7.45 (m, 2H), 6.70 (m, 1H), 6.01 (d, *J* = 16.0 Hz, 1H), 5.86 (m, 1H), 5.65 (m, 1H), 5.28 (m, 1H), 5.12 (d, *J* = 8.0 Hz, 1H), 4.71 (m, 2H), 4.64 (m, 2H), 4.00 (d, *J* = 4.0 Hz, 1H), 3.67 (m, 1H), 3.46 (m, 3H), 3.26 (s, 3H), 3.24 (s, 3H), 2.32 (m, 2H), 2.12 (m, 1H), 1.62 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 166.2, 148.1, 142.1, 135.4, 133.0, 130.5, 130.2, 129.3, 128.5, 127.8, 126.0,

119.6, 93.6, 85.7, 74.8, 73.8, 71.7, 68.1, 61.6, 57.9, 56.5, 53.5, 38.0, 35.6, 31.7, 24., 18.1 ppm. MS (ESI): *m/z* 537 $[M+Na]^+$ 553 $[M+K]^+$. Anal. ($C_{29}H_{38}O_8$) C, H, N.

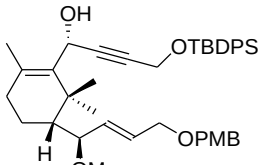
Synthesis of aldehyde 22



22

A solution of silyl ether **9** (620 mg, 1.01 mmol) in THF (5 mL) was treated with TBAF and the resulting solution was stirred at 40 °C for 14h. The mixture was then allowed to reach rt and concentrated under vacuum. The crude material was filtered through a pad of silica gel (hexanes/Et_2O 1:3) affording the corresponding primary alcohol as a colorless oil in 93% yield. Primary alcohol was directly used in the next step. Tetra(*n*-propyl)ammonium perruthenate (5 mg, 0.015 mmol) was added portionwise to a suspension of 115 mg (0.31 mmol) of primary alcohol intermediate in CH_2Cl_2 (4 mL), molecular sieves 4Å (50 mg) and 4-methylmorpholine-N-oxide (43 mg, 0.37 mmol) at 0 °C. The reaction mixture was stirred for 2h at rt and then filtered through a Celite pad. The filtrate was concentrated under reduced pressure and purified by flash chromatography (hexanes/Et_2O 1:2) to afford the aldehyde **22** as colorless oil in quantitative yield. ^{1}H-NMR (200Mz, $CDCl_3$) δ: 10.09 (s, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 5.68 (m, 2H), 4.45 (s, 2H), 4.02 (q, *J* = 15.6, 4.4 Hz, 2H), 3.81 (brs, 1H), 3.79 (s, 3H), 3.17 (s, 3H), 2.20 (m, 2H), 2.07 (s, 3H), 1.72-1.53 (m, 3H), 1.27 (s, 3H), 1.17 (s, 3H) ppm. ^{13}C-NMR (100MHz, $CDCl_3$) δ: 192.2, 159.1, 155.5, 140.6, 132.8, 130.1, 129.2, 129.4, 127.8, 113.6, 79.5, 71.6, 69.7, 55.9, 55.0, 50.8, 36.5, 35.8, 26.5, 21.2, 19.3, 17.5 ppm. IR ($CHCl_3$) (*v*, cm^{-1}): 2937, 1668, 1613, 1514. MS (ESI): *m/z* 395.1 $[M+Na]^+$. Anal. ($C_{23}H_{32}O_4$) C, H, N.

Synthesis of alcohol 23



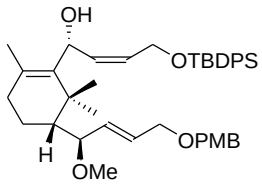
23

A solution of O-TBDPS–propargyl alcohol[12] (130 mg, 0.77 mmol) in THF (3 mL) was cooled to -78 °C and treated with a 1.6 M solution of *n*BuLi (0.64 mL, 1.02 mmol). The reaction mixture was stirred at -78 °C for 30 min. A solution of the aldehyde **22** (190 mg, 0.51 mmol) in THF (2 mL) was

added to the first solution at -78 °C. The resulting reaction mixture was stirred at -20 °C for 2h and then left to reach rt over night. The solution was then diluted with Et_2O (15 mL) and aqueous NH_4Cl (20 mL) was added. Mixture was extracted twice with Et_2O (10 mL). The organic phases were collected, dried (Na_2SO_4), concentrated and purified by flash chromatography (Et_2O/petroleum ether 1:2) to afford compound **23** as colorless oils in 90% overall yield.

^{1}H-NMR (400Mz, CD_2Cl_2) δ: 7.68 (m, 4H), 7.39 (m, 6H), 7.24 (d, , *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 5.68 (m, 2H), 4.97 (s, 1H), 4.41(s, 2H), 4.37 (d, *J* = 1.7 Hz, 2H), 4.02 (d, *J* = 5.0 Hz, 2H), 3.77 (s, 3H), 3.74 (d, *J* = 6.0 Hz, 1H), 3.17 (s, 3H), 1.95 (m, 2H), 1.80 (s, 3H), 1.63-1.40 (m, 3H), 1.12 (s, 3H), 1.02 (s, 9H), 0.91 (s, 3H) ppm. ^{13}C-NMR (100MHz, CD_2Cl_2) δ: 159.0, 138.1, 135.5, 134.6, 133.0, 130.6, 129.7, 129.2, 127.9, 113.6, 86.8, 82.1, 80.4, 71.6, 69.9, 65.6, 59.7, 55.7, 55.1, 50.5, 38.2, 33.6, 26.2, 22.0, 20.9, 18.9, 18.2, 15.0 ppm. IR (CHCl3) (*v*, cm-1): 3009, 2933, 1613, 1514. MS (ESI): *m/z* 689.4 $[M+Na]^+$. Anal. ($C_{42}H_{54}O_5Si$) C, H, N.

Synthesis of alkene 24

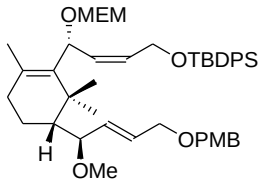


24

Quinoline (82.5 μL, 0.7 mmol) and Lindlar's catalyst (470 mg) were added to a solution of alkyne **23** (470 mg, 0.7 mmol) in ethyl acetate (40 mL). The suspension was stirred under hydrogen atmosphere for 2h then filtered through a Celite pad. The filtrate was concentrated and purified by flash chromatography (Et_2O/petroleum ether 1:2) to give *Z*-olefine **24** as colorless oil in 80% yield.

^{1}H-NMR (400Mz, CD_2Cl_2) δ: 7.69 (m, 4H), 7.41 (m, 6H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 5.78-5.58 (m, 4H), 4.89 (d, *J* = 6 Hz, 1H), 4.40 (s, 4H), 4.00 (d, *J* = 5.0 Hz, 2H), 3.77 (s, 3H), 3.70 (d, *J* = 6.0 Hz, 1H), 3.15 (s, 3H), 1.98-1.80 (m, 3H), 1.74 (s, 3H), 1.56 (m, 2H), 1.09 (s, 3H), 1.04 (s, 9H), 0.79 (s, 3H) ppm. ^{13}C-NMR (100MHz, CD_2Cl_2) δ: 159.2, 140.0, 135.5, 134.8, 133.5, 132.9, 130.6, 129.7, 129.3, 127.8, 127.7, 113.6, 80.5, 71.6, 71.5, 71.2, 69.9, 66.8, 60.8, 55.7, 55.1, 50.7, 38.4, 33.8, 26.7, 26.5, 21.9, 20.9, 18.3 ppm. IR ($CHCl_3$) (*v*, cm-1): 3350, 3007, 2934, 1613, 1514; MS (ESI): *m/z* 691.0 $[M+Na]^+$. Anal. ($C_{42}H_{56}O_5Si$) C, H, N.

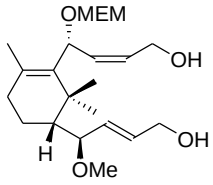
Synthesis of MEM-derivative 25



25

To a solution of alcohol **24** (290 mg, 0.43 mmol) in dry CH_2Cl_2 (6 mL), DIPEA (1 mL, 5.2 mmol), MEMCl (245 μl, 2.15 mmol) and Bu_4NI (0.002 mmol) were added. The reaction mixture was stirred at 40 °C for 2h. After cooling to rt the organic phase was diluted with CH_2Cl_2 (10 mL), washed with water (10 mL), concentrated and dried. The crude mixture was purified by flash chromatography (Et_2O/petroleum ether 1:3) to afford compound **25** as colorless oil in 75% yield. ^{1}H-NMR (400Mz, CD_2Cl_2) δ: 7.70 (m, 4H), 7.43 (m, 6H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 5.67 (m, 4H), 4.87 (d, *J* = 8.4 Hz, 1H), 4.53(m, 2H), 4.43 (m, 2H), 4.42 (s, 2H), 4.02 (d, *J* = 4.8, 2H), 3.79 (s, 3H), 3.54 (d, *J* = 6.0 Hz, 1H), 3.47-3.34 (m, 4H), 3.24 (s, 3H), 3.16 (s, 3H), 1.92 (m, 2H), 1.74 (s, 3H), 1.56-1.42 (m, 3H), 1.10 (s, 3H), 1.05 (s, 9H), 0.78 (s, 3H) ppm. ^{13}C-NMR (100MHz, CD_2Cl_2) δ: 159.2, 136.7, 135.5, 133.7, 133.2, 132.1, 130.6, 129.8, 129.6, 129.8, 127.6, 113.6, 91.6, 80.6, 71.6, 69.9, 69.2, 66.9, 65.6, 60.2, 58.5, 55.1, 50.8, 38.3, 33.9, 27.0, 26.5, 20.9, 18.9, 18.4 ppm. IR (CHCl3) (*v*, cm-1): 2934, 1613; MS (ESI): *m/z* 778.9 $[M+Na]^+$. Anal. ($C_{46}H_{64}O_7Si$) C, H, N.

Synthesis of diol 26

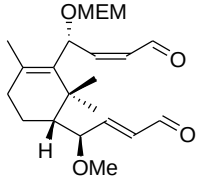


26

A solution of *p*-methoxybenzyl ether **25** (300 mg, 0.396 mmol) in CH_2Cl_2/phosphate buffer (14 mL : 1.4 mL) was treated with DDQ (90 mg, 0.396 mmol) and the resulting solution was stirred at rt for 30 min. The reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash chromatography (AcOEt/hexanes 1:2) to give the corresponding primary alcohol (244 mg) as yellow oil in 97% yield. This latter compound (300 mg, 0.471 mmol) was immediately dissolved in dry THF (2 mL) and TBAF (2.35 mmol) was added. The mixture was heated at 40 °C. After 1h the reaction mixture was cooled to rt, concentrated and then purified by flash chromatography (AcOEt/hexanes 1:1) to give the diol **26** as colorless oil in quantitative yield.

^{1}H-NMR (400Mz, CD_2Cl_2) δ: 5.67-5.50 (m, 4H), 5.07 (m, 1H), 4.72 (d, *J* = 6.8 Hz, 1H), 4.59 (d, *J* = 6.8 Hz, 1H), 4.49-4.36 (m, 1H), 4.02-3.94 (m, 4H), 3.72-3.45 (m, 4H), 3.27 (s, 3H), 3.07 (s, 3H), 2.58 (br s, 2H), 1.84-1.49 (m, 4H), 1.38-1.14 (m, 2H), 1.08-0.73 (m, 2H), 1.03 (s, 3H), 0.73 (s, 3H) ppm. ^{13}C-NMR (100MHz, CD_2Cl_2) δ: 134.2, 133.0, 132.4, 129.8, 129.4, 126.8, 95.6, 88.6, 71.2, 66.5, 65.8, 62.3, 59.7, 59.3, 54.3, 53.8, 38.3, 31.9, 26.5, 17.3, 15.4 ppm. MS (ESI): *m/z* 421 $[M+Na]^+$. Anal. ($C_{22}H_{38}O_6$) C, H, N.

Synthesis of dialdehyde 27

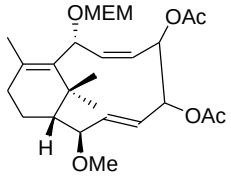


27

Tetra(*n*-propyl)ammonium perruthenate (7.4 mg, 0.022 mmol), molecular sieves 4Å (140 mg) and 4-methylmorpholine-N-oxide (221 mg, 1.88 mmol) were added portionwise at 0 °C to a suspension of diol **26** (187 mg, 0.47 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was allowed to reach rt and was then stirred for 30 min. Then the reaction mixture was filtered through a Celite pad. The filtrate was concentrated and purified by flash chromatography (AcOEt/hexanes 1:1) to afford the dialdehyde **27** as colorless oil in quantitative yield.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 10.30 (d, *J* = 8.0 Hz, 1H), 9.54 (d, *J* = 8.0 Hz, 1H), 6.60 (m, 2H), 6.15 (m, 1H), 5.85 (m, 1H), 5.57 (m, 1H), 4.66 (m, 2H), 4.03 (s, 1H), 3.57 (s, 2H), 3.45 (s, 2H), 3.29 (s, 3H), 3.17 (s, 3H), 1.94 (m, 3H), 1.70 (s, 3H), 1.48 (m, 2H), 1.21 (s, 3H), 0.98 (s, 3H), MS (ESI): *m/z* 395 $[M+H]^+$. Anal. ($C_{22}H_{34}O_6$) C, H, N.

Synthesis of carbocycle 7



7

$TiCl_4$ (111 µL, 0.8 mmol) was dissolved into dry THF (16 ml) at 0 °C. After warming to rt, Zn dust (104 mg, 1.6 mmol) and pyridine (65 µL, 0.8 mmol) were added. A solution of dialdehyde **27** (15 mg, 0.04 mmol) in dry THF (2 mL) was then added dropwise via syringe pump over 2h, and the mixure was stirred for an additional 30 minutes. The reaction was then treated with 10% aqueous

K_2CO_3 (10 mL). The mixture was extracted twice with Et_2O (15 mL). The organic phases were collected, dried over Na_2SO_4, concentrated and purified by flash chromatography (AcOEt 100%) to afford compound **28** as colorless oil in 49% yield.

Compound **28** (3 mg, 0.0075 mmol) was then dissolved in dry DCM (3 mL), and Et_3N (13 μL, 0.09 mmol), DMAP (catalytic amount) and Ac_2O (7 μL, 0.075 mmol) were added at 0 °C. The reaction mixture was stirred at rt for 1h. An aqueous solution of $NaHCO_3$ (5 mL) was then added and the mixture was extracted with AcOEt (5 mL) twice. The organic phases were collected, dried over Na_2SO_4, concentrated and purified by flash chromatography (AcOEt 100%) affording the desired compound **7** in quantitative yield. Data of major diastereoisomer were reported.

^{1}H-NMR (400MHz, $CDCl_3$) δ: 6.09 (m, 1H), 5.65 (m, 1H), 5.46 (m, 1H), 5.30 (m, 1H), 5.18 (m, 1H), 4.61 (m, 1H), 4.03 (m, 1H), 3.49 (m, 1H), 3.40 (q, *J* = 8Hz, 4H), 3.31 (m, 5H), 3.13 (s, 3H), 2.03 (m, 8H), 1.71 (m, 3H), 1.49 (s, 3H), 1.18 (s, 3H), 1.06 (s, 3H) ppm. ^{13}C-NMR (100MHz, CD_2Cl_2) δ: 171.7, 171.3, 133.8, 133.1, 132.4, 128.8, 128.0, 126.8, 94.8, 82.6, 78.1, 74.7, 74.7, 64.4, 62.3, 59.6, 57.6, 56.8, 38.3, 34.6, 26.9, 26.4, 23.6, 21.0, 18.2, 16.7 ppm. MS (ESI): *m/z* 481 $[M+H]^+$. Anal. ($C_{26}H_{40}O_8$) C, H, N.

References

1. Mohamadi, F.; Richards, M. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440-467.
2. Kaminski, G. A.; Friesner, R. A.; Tirado-Rives, J.; Jorgensen, W. L. *J.Phys.Chem.B* **2001**, *105*, 6474-6487.
3. DiscoveryStudio, version 2.5 **2009**.
4. Sako, M.; Suzuki, H.; Hirota, K. *Chem.Pharm. Bull* 1998, *46*, 1135-1139.
5. Neri A. *et al.*, Inhibition of P-glycoprotein-mediated transport of rhodamine 123 by novel N,N-bis(cyclohexanol)amine aryl esters: structure-activity relationships. *Submitted*
6. Pastan I, Gottesman MM, Ueda K, Lovelace E, Rutherford AV, Willingham MC. A retrovirus carrying an MDR1 cDNA confers multidrug resistance and polarized expression of P-glycoprotein in MDCK cells. *Proc Natl Acad Sci U S A* **1988**, *85*, 4486-90.

7. Weaver JL, Szabo G Jr, Pine PS, Gottesman MM, Goldenberg S, Aszalos A. The effect of ion channel blockers, immunosuppressive agents, and other drugs on the activity of the multi-drug transporter. *Int J Cancer* **1993**; 54:456-61.

8. Kobayashi, J.; Hosoyama, H.; Wang, X. X.; Shigemori, H.; Sudo, Y.; Tsuruo, T. Modulation of multidrug resistance by taxuspine C and other taxoids from Japanese yew. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1555-8.

9. Sako, M.; Suzuki, H.; Hirota, K. Syntheses of taxuspine C derivatives as functional inhibitors of P-glycoprotein, an ATP-associated cell-membrane transporter. *Chem. Pharm. Bull.* **1998**, *46*, 1135-9.

10. Kobayashi, J.; Shigemori, H.; Hosoyama, H.; Chen, Z.; Akiyama, S.; Naito, M.; Tsuro, T. Multidrug Resistance Reversal Activity of Taxoids from Taxus cuspidata in KB-C2 and 2780AD Cells. *Jpn. J. Cancer Res.* **2000**, *91*, 638-42.

11. Avramova, S. I.; Galletti, E.; Renzulli, M. L.; Giorgi, G.; Sgaragli, G.; Alderighi, D.; Ghiron, C.; Corelli, F.; Radi, M.; Botta, M. *ChemMedChem* **2008**, *3*, 745-748.

12. Prepared accordingly to literature procedure: propargyl alcohol (1 eq.), TBDPSiCl (1.2 eq.), imidazole (1.6 eq.), DMF, room temperature, 12 h.