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Synthesis of an orthogonally protected polyhydroxylated cyclopentene from L-sorbose

Daniele Lo Rea*, Leigh Jonesa, Ernest Giraltb,c and Paul Murphya*

Dedicated to Mª Teresa Plaza López-Espinosa on the occasion of her retirement (optional)

Abstract: The use of L-sorbose in synthesis of functionalized cyclopentene derivatives was accomplished. These cyclopentene derivatives are related to those found in naturally occurring jatrophone frameworks and in other bioactive compounds. The formation of allyl α-L-sorbopyranoside was a key synthetic step. Regioselective introduction of protecting groups was followed by the hydrolysis of the allyl glycoside to furnish a fully protected acyclic L-sorbose derivative. This acyclic intermediate was subsequently used to give an orthogonally protected polyhydroxylated cyclopentene, which has potential in further bioactive compound synthesis. The protected cyclopentene itself showed a clear cytotoxic activity when tested against a panel of human cancer cell lines (HT29, LS174T, SW620, A549, HeLa cells).

The occurrence of resistance to anticancer agents is a major obstacle for successful cancer chemotherapy. The emergence of resistance to anticancer drugs, in particular multidrug resistance (MDR) has made many of the available anticancer drugs ineffective.[1] A glycoprotein, ABCB1, also known as P-glycoprotein, is a membrane protein member of the ABC transporters superfamily. These membrane-embedded transport proteins decrease the intracellular drug accumulation, by and ATP-dependent efflux. This reduces the cytotoxicity of the anticancer agent and enables the tumor cells to survive. Jatrophanes, such as 1-3, are natural compounds extracted from plants of the genus of Euphorbia. A broad range of biological properties have been reported for constituents of the plant extract.[2] In particular, jatrophanes are potent and specific P-glycoprotein modulators.[3] In addition, a variety of other biological activities have been reported: inhibitory activity on the mammalian mitochondrial respiratory chain,[4] antiviral activity,[5] microtubole interaction,[6] antiplasmodial activity,[7] cytotoxicity against various human cancer cell lines.[8]
It has been reported that modifications of the cyclopentene (ring A) of the jatrophane framework, resulted in increased biological activity (figure 1). For instance, Hierseman and co-workers demonstrated that introduction of aromatic lipophilic substituent at C-3 enhanced the ABCB1 inhibitory activity (figure 1). They also show that there is a correlation between overall lipophilicity and ABCB1 inhibition.

Figure 1. a) Structure of selected jatrophane natural products; b) synthetic jatrophane analogues and their activity against ABCB1; c) orthogonally protected pyranose and open-chain sorbose derivatives and the orthogonal protected cyclopentene prepared herein.

Nevertheless, the potential to prepare cyclopentene derivatives related to those found in 4 and which would ultimately be used to give new jatrophone analogues has formed the motivation for the work described herein. We envisaged that the preparation of orthogonally protected polyhydroxylated cyclopentanol, similar to the ring A of the jatrophane framework, is relevant to the preparation of jatrophone analogues with increased biological activity and for structure-activity relationships. In addition, advanced intermediate can be also used for the
preparation of biologically active cyclopentitols or cyclopentenols. These are polyhydroxylated
cyclopentanes,\textsuperscript{[10]} that display a plethora of biological activity such as found for the
glycosidase inhibitors trehazolin and mannostatin,\textsuperscript{[10a]} the anticancer and antibiotic
pactamycin,\textsuperscript{[12]} and the insecticide ryanodine.\textsuperscript{[13]} In addition, cyclopentenols, are also important
because of their presence in numerous biologically active targets such as the antibiotic
pentenomycin,\textsuperscript{[14]} the anti-inflammatory monotropein,\textsuperscript{[15]} and the anticancer compound (-)-
neplanocin A.\textsuperscript{[16]}

It was envisaged that the construction of the polyhydroxylated cyclopentene could be achieved
through a ring-closing metathesis\textsuperscript{[10c, 17]} (RCM) reaction of fully protected diene such as that in
11. Since RCM could be challenging in such a densely substituted diene,\textsuperscript{[18]} we first prepared
11 in order to validate the synthetic strategy.

\textbf{Scheme 1.} Preliminary validation of the synthetic strategy from L-sorbose: synthesis of per-O-
benzylated cyclopentane 12.

Compound 9 has been prepared from L-sorbose previously.\textsuperscript{[19]} However, in our hands,
attempts to simultaneously install olefin at C6 and C2 through directly oxidation of 9 followed
by a concomitant double Wittig reaction were unsuccessful. Oxidation of the latent alcohol of
9 under Swern conditions followed by Wittig reaction only gave a complex mixture while
treatment of 9 with the Dess-Martin reagent gave no reaction. For these reasons, aldehyde 10
was first prepared as reported\textsuperscript{[19]} and then treated with Ph\textsubscript{3}PCH\textsubscript{2}Br and tBuOK to give the
desired diene 11. The RCM of 11 using the Grubbs 2\textsuperscript{nd} generation catalyst at room
temperature only gave only recovered starting material. However, when the RCM was carried
out by heating in refluxing toluene, the polyhydroxylated cyclopentene 12 was isolated in good
yield (76%, Scheme 1).

With these encouraging observations in hand, it was next decided to exploit this strategy for
the preparation of an orthogonally protected L-sorbose derivative. Chemical manipulation of
L-sorbose is often carried out via its methyl L-sorbopyranoside\textsuperscript{[19]} or its 2,3:4:6-di-O-
isopropyliden-α-L-sorbofuranose. However, these synthetic approaches suffer drawbacks. In fact, hydrolysis of the dioxolane ring (for the L-sorbofuranose) or hydrolysis of the methyl glycoside (for the L-sorbopyranoside) requires strong acidic conditions, which are not compatible with several functional or protecting groups. On the other hand, the allyl group can be removed in the presence of different protecting groups under relatively mild conditions. Since the synthesis of the L-sorbopyranoside 8 is achieved using MeOH in the presence of acetyl chloride (AcCl), we hypothesized that similar conditions could be used for the preparation of the allyl glycoside 13. In fact, treatment of L-sorbose with allyl alcohol and AcCl gave 13 in 64% yield. However, at this stage we could not establish the stereochemical configuration of 13. However, 1H-NMR analysis suggested the formation of the pyranose ring \( J_{3,4} = 9.5 \text{ Hz}; J_{5,6} = 10.4; J_{4,5} = 8.7; J_{5,6'} = 5.4 \) had occurred. Fortunately, regioselective protection of hydroxyl groups at C-1 and C-3 using anisaldehyde dimethyl acetal in presence of camphorsulphonic acid gave 14 in good yield (scheme 2) which crystallized from a mixture of MeOH-AcOEt 3:1. X-Ray crystal structure determination of the resultant crystals confirmed the formation of the allyl α-L-sorbopyranoside 14 as the main product (see SI). The regioselective protection of diol 14 was next attempted. Unfortunately, the attempted reaction of 14 with TIPSCI in pyridine was not successful. Similar results were obtained when 14 was treated with MOMCl in pyridine. Fortunately, regioselective protection of 14 was achieved using MOMCl and DIPEA in dichloromethane, giving alcohol 15 in moderate yield (55%), together with small amounts of 16 (14%) and a mixture of regioisomers 15 and 17 (16%), after chromatography. We next attempt the protection of the secondary alcohol of 15 as the TBS ether. However, the introduction of the TBS group failed under several conditions (TBSOTf-TEA-THF, TBSOTf-NaH-THF, TBSOTf-DBU, TBSCl-NaH-DMF). Fortunately, treatment of 15 with benzoyl chloride in pyridine gave 18 in excellent yield (93%). The analysis of NMR data for 19 confirmed that the hydroxyl group at C-4 was protected as the MOM ether while the hydroxyl group at C-5 was protected as the benzoate ester. In addition, the mixture of 15 and 17 obtained during regioselective MOM protection of 14 was reacted with BzCl in pyridine giving both 18 and 21 that can be easily separated using chromatography. Treatment of the 3,6-O-p-methoxybenzylidene acetal 18 with NaCNBH 3 and TMSI gave the corresponding 4-O-PMB ether 19 in 59% yield.

Next, the protection of the secondary hydroxyl group of 19 was attempted. Unfortunately, protection of 19 as a TIPS ether was unsuccessful after several attempts (using TIPSCI or TIPSOTf, DMAP, DMF, 130 °C or TIPSOTf, NaH, THF, RT), with 19 being recovered unreacted. Surprisingly, even benzylation of 19 failed under several conditions (NaH and BnBr...
or Dudley’s reagent\textsuperscript{(26)}). Instead, removal of the allyl group, followed by installation of the protecting group at C-3 were studied. However, attempted removal of the allyl protecting group using PdCl\textsubscript{2} at several different conditions gave the desired product in low yields (28-41\%). Fortunately, treatment of 19 with OsO\textsubscript{4}/NaIO\textsubscript{4}\textsuperscript{[23b]} followed by the simultaneous protection of both the C-3 and C-6 OH groups as TBS ethers gave 20 in good yield (58\% over two steps). With 20 in hand, chain branching at C-2 using the Wittig reaction was investigated. Treatment of 20 with Ph\textsubscript{3}PCH\textsubscript{3}Br and tBuOK gave the desired alkene 22 in 61\% yield. Regioselective removal of the TBS group at the primary position of 22 using 10 \% TsOH in MeOH gave a primary alcohol, which was oxidized using Ley-Griffith conditions and gave aldehyde 23 in good yield. Again, compound 23 was reacted with Ph\textsubscript{3}PCH\textsubscript{3}Br and tBuOK to give diene 24 (Scheme 4). However, the reaction of 24 with the Grubbs 2\textsuperscript{nd} generation catalyst in toluene only gave a complex mixture at different temperatures (from 80 °C to reflux).

Fortunately, RCM of 24 in dichloromethane under microwave irradiation gave the orthogonally protected cyclopentene 25 in good yield (71\%). Removal of PMB group in 25 was successful using DDQ-H\textsubscript{2}O leading to 26 (66\%). Treatment of 26 with MnO\textsubscript{2} gave the α,β-unsaturated aldehyde 27 in 74\% yield. Aldehyde 27 was then reacted with isopropenylmagnesium bromide to give secondary alcohol 28 (72\%) that was finally oxidized with MnO\textsubscript{2} giving the orthogonally protected advanced intermediate 7 in 93\% yield (Scheme 3).

Importantly, advanced intermediate 7 has potential to be used for the preparation of analogues of MDR modulators such as jatrophanes and can be also used for the preparation of biologically active cyclopentitols. In addition, intermediate 7 was screened against a panel of human cancer cell lines derived from colon (HT29, Ls174t, SW620), lung (A549) and cervical cancer (HeLa). We took advantage that 7 has a silyl ether, a feature that could increase cellular uptake and enhance anti-proliferative effects. In addition, It has also been demonstrated that no “element-specific” toxicity is associated with organosilicon compounds.\textsuperscript{[27]} Padron and co-workers have demonstrated that the presence of TBS ether enhance the anti-proliferative effects of tetrahydropyrans derivatives in HL60 (human promyelocytic leukemia) and MCF7 (human breast cancer) cell lines.\textsuperscript{[28]} Furthermore, trialkysilyl camptothecine derivatives such as cositecan\textsuperscript{[29],[30]} and DB-67\textsuperscript{[31]} are promising compounds for cancer treatment and are currently in phase III and II clinical trials respectively.
Scheme 2. a) Synthesis of open-chain intermediate 20; b) resolution of 15-17 mixture; c) crystal structure of 14.

Compound 7 showed good toxicity in the XTT assay with IC\textsubscript{50} range of 6.1-35.1 µM. Compound 7 is generally less potent than widely used chemotherapeutics cisplatin or oxaliplatin while in HT29 colon cancer cell line, displayed an activity similar to cisplatin. Previous studies have demonstrated the ability of jathropane diterpenes to reduce the activity of ABC proteins linked with multi-drug resistance (MDR).\textsuperscript{[32]} Compound 7 can be considered as an analogue of ring A of jathropane (figure 1). In light of that, we decided to test 7 against ABCC2 (MRP2), ABCC3 (MRP3) and ABCB1 (MDR1 or P-gp). At the concentration of 20 µM, compound 7 show weakly inhibit ABCC2 (10.5 ± 0.25%), ABCC3 (6.1± 0.35%) and ABCB1 (7.1± 0.65%) indicating that truncated\textsuperscript{[33]} jatrophane analogues might have potential to be developed into MDR modulators.

In conclusions, we have developed a synthetic approach for the conversion of L-sorbose to allyl α-L-sorbo-pyranoside (13). This intermediate was turned into an orthogonally protected L-sorbo-pyranose derivative (18). The cleavage of the allyl glycoside in 19 is compatible with different protecting group (acidic, basic and single electron oxidation sensitive). Subsequently, we also prepared the orthogonal acyclic L-sorbose derivative (20): this intermediate can be used for the preparation of more complex chiral products. We demonstrated its use in preparation of the orthogonally protected polyhydroxylated cyclopentene 7 which could have potential as an intermediate for the preparation of jatrophane analogues or for the preparation of cyclopentitols which are of particular significance because of their presence in a variety of medicinally relevant natural products. Compound, 7 was tested in a panel of human cancer cell lines (HT29, Ls174t, SW620, HeLa, A549) and displayed good cytotoxic activity. In addition, 7 weakly inhibit ABCB1 ABCC2 and ABCC3 demonstrating that it could be used as a starting point for the development of truncated jatrophane analogues as multi drug resistance (MDR) modulators.

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<th>HT29</th>
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<th>SW620</th>
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Figure 2. Antiproliferative activity of 7: IC_{50} was determined by XTT assay. a: see ref \cite{34}
**General methods and materials.** All NMR spectra were recorded using a 500 MHz spectrometer at 30 °C. Chemical shifts are reported relative to internal Me$_4$Si in CDCl$_3$ (δ 0.0 ppm) for $^1$H and CDCl$_3$ (δ 77.16) for $^{13}$C at 30 °C, unless otherwise stated. The $^{13}$C signals were assigned with the aid of HSQC. The $^1$H-NMR signals were assigned with the aid of COSY. Coupling constants are reported in Hertz. High resolution mass spectra were measured using an LC Time-of-flight mass spectrometer and were measured in positive and/or negative mode as indicated. TLC were performed on aluminium sheets precoated with Silica Gel 60 (HF254, E. Merck) and spots visualized charring with vanillin solutions. Flash column chromatography was carried out using silica gel 60 (0.040-0.630 mm, E. Merck). Dichloromethane, tetrahydrofuran, MeOH, and toluene were used as obtained from a PureSolv™ solvent purification system. Petroleum ether is the fraction with bp 40-60 °C.

**Allyl 1,3-O-(4-methoxybenzylidene)-α-L-sorbopyranoside (14).** To a stirred solution of 13 (2.364 g; 10.7 mmol) in DMF (23 mL), camphorsulphonic acid (248 mg; 1.07 mmol) and anisaldehyde dimethyl acetal (1.82 mL; 10.7 mmol) were added. The solution was stirred at room temperature for 5 h. The mixture was diluted with Et$_2$O (30 mL) and washed several times with water. The solvent was evaporated and flash chromatography (EtOAc-petroleum ether 3:1) gave 14 as a white solid (2.91 g; 80%); m.p. = 104 °C-109 °C; [α]$_D$ - 41 (c 0.69, CHCl$_3$); $^1$H NMR (CDCl$_3$, 500 MHz): δ 7.48 – 7.36 (d, $J = 8.8$ Hz, 2H, ArCH), 6.91 – 6.82 (d, $J = 8.7$ Hz, 2H, ArCH), 5.99 (ddt, $J_{trans} = 16.9$, $J_{cis} = 10.5$, 5.3 Hz, 1H, OCH$_2$CH=CH$_2$), 5.51 (s, 1H, OCHO), 5.37 (dd, 1H, $J = 17.3$, $J_{gem} = 1.7$ Hz, OCH$_2$CH=CH$_2$), 5.18 (dd, 1H, $J_{cis} = 10.6$, OCH$_2$CH=CH$_2$), 4.26 (dd, 1H, $J = 12.1$, 2.0 Hz, CH$_2$), 4.18 – 4.11 (m, 1H, OCH$_2$CH=CH$_2$), 3.79 (s, 3H, OCH$_3$), 3.75 – 3.62 (m, 2H, CH$_2$, CH), 3.58 – 3.49 (m, 2H, CH$_2$), 3.44 (dd, 1H, $J = 9.8$, 2.7 Hz, CH); $^{13}$C NMR (CDCl$_3$, 125 MHz): 160.4 (ArC), 134.4 (OCH$_2$CH=CH$_2$), 129.7 (ArC), 128.0, (ArCH), 116.7 (OCH$_2$CH=CH$_2$), 113.8 (ArCH), 102.7 (OCHO), 92.8 (C-2), 82.0 (CH), 71.2 (CH), 70.7 (CH), 68.2 (CH$_2$), 63.3 (CH$_2$), 62.0 (CH$_2$), 55.5 (OCH$_3$); HRMS-ESI: calcd for C$_{17}$H$_{22}$O$_7$Na: 361.1263; Found: 361.1266 (M+Na$^+$).

**Allyl 5-O-benzoyl-1,3-O-(4-methoxybenzylidene)-4-O-(methoxymethoxy)-α-L-sorbopyranoside (18).** To a stirred solution of 15 (430 mg; 1.12 mmol) in pyridine (10 mL) at 0 °C, BzCl (195 µL; 1.68 mmol) was added. The mixture was stirred at room temperature for 18 h. TLC (petroleum ether-EtOAc, 2:1) showed a new spot with higher R$_f$ and the absence of
15. The solution was diluted with dichloromethane (20 mL), and washed with 1% HCl (20 mL), dried over Na$_2$SO$_4$, filtered and the solvent was removed. Flash chromatography (cyclohexane-EtOAc, 5:1) gave 18 as a yellow oil (508 mg; 93%); [α]$_D^20$ -17.16 (c 0.85, CHCl$_3$); $^1$H NMR (CDCl$_3$, 500 MHz): 8.09 (m, 2H, ArCH), 7.60–7.55 (m, 1H, ArCH), 7.47–7.43 (m, 4H, ArCH), 6.89 (d, 2H, $J$ = 8.8 Hz, ArCH), 6.03 (ddt, 1H, $J_{\text{trans}}$ = 17.3, $J_{\text{cis}}$ = 10.6, 5.4 Hz, OCH$_2$CH=CH$_2$), 5.59 (s, 1H, OCH$_2$), 5.40 (dd, 1H, $J_{\text{trans}}$ = 17.2, $J$ = 1.7 Hz, OCH$_2$CH=CH$_2$), 5.27–5.16 (m, 2H, OCH$_2$CH=CH$_2$, H-5), 4.84 (d, 1H, $J$ = 6.8 Hz, OCH$_2$O), 4.67 (d, 1H, OCH$_2$O), 4.47 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 4.32 (d, 1H, $J_{1,1'} = 12.1$ Hz, H-1), 4.21 (ddt, 1H, $J$ = 12.7, 5.1, 1.6 Hz, OCH$_2$CH=CH$_2$), 4.08 (ddt, 1H, $J$ = 12.7, 5.1, 1.6 Hz, OCH$_2$CH=CH$_2$), 4.01 (dd, 1H, $J_{6,6'} = 10.6$, $J_{6,5} = 6.4$ Hz, H-6), 3.81 (s, 3H, ArOC$_3$H), 3.74 (d, 1H, H-3), 3.63 – 3.61 (m, 2H, H-1'), 3.06 (s, 3H, OCH$_2$OCH$_3$); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 165.8 (CO), 160.3 (ArC), 134.4 (OCH$_2$CH=CH$_2$), 133.5 (ArCH), 129.9 (ArC), 129.9 (ArCH), 129.6 (ArC), 128.6 (ArCH), 127.8 (ArCH), 116.8 (OCH$_2$CH=CH$_2$), 113.7 (ArCH), 102.4 (OCHO), 97.3 (OCH$_2$O), 93.0 (C-2), 82.1 (C-3), 73.0 (C-4), 71.7 (C-5), 68.1 (C-1), 62.3 (OCH$_2$CH=CH$_2$), 60.9 (C-6), 55.8 (OCH$_2$OCH$_3$), 55.4 (ArOCH$_3$); HRMS-ESI: calcd for C$_{26}$H$_{30}$O$_9$Na: 509.1788; Found: 509.1788 (M+Na$^+$).

5-O-benzoyl-3,6-di-O-(Tert-butyldimethylsilyl)-1-O-(4-methoxybenzyl)-4-O-(methoxymethoxy)-L-sorbose (20). To a stirred solution of 19 (657 mg; 1.34 mmol) in dioxane (6.5 mL) and H$_2$O (0.7 mL), NMO (473 mg; 4.039 mmol), OsO$_4$ (1.419 mL; ~0.134 mmol, 2.5% in t-BuOH), NaIO$_4$ (1.146 g; 5.36 mmol) and H$_2$O (2.6 mL) were added. The resulting solution was stirred at 60 °C for 24 h. TLC (petroleum ether-EtOAc 1:1) showed a new spot with lower R$_f$ and the absence of 19. The mixture was diluted with dichloromethane and washed with H$_2$O. The organic layers were washed with aq satd Na$_2$S$_2$O$_3$. The organic phase was dried and the solvent was removed. Flash chromatography (cyclohexane-EtOAc, 30:1 to 10:1 gradient elution gave 20 as a yellow oil (525 mg; 58% over two steps); [α]$_D^20$ -19.6° (c 0.41, CHCl$_3$); $^1$H NMR (CDCl$_3$, 500 MHz): δ 8.03–7.97 (m, 2H, ArCH), 7.55–7.50 (m, 1H, ArCH), 7.41 (t, 2H, $J$ = 7.8 Hz, ArCH), 7.09 (d, 2H, $J$ = 8.6 Hz, ArCH), 6.79 (d, 2H, $J$ = 8.6 Hz, ArCH), 5.39 (td, 1H, $J$ = 5.8, 4.3 Hz, H-5), 4.75 (d, 2H, $J$ = 1.6 Hz, CH$_2$), 4.50 (d, 1H, $J$=5.1 Hz, H-3), 4.34 – 4.24 (m, 2H, CH$_2$), 4.01 (dd, 1H, $J_{6,6'} = 10.6$, $J_{6,5} = 6.4$ Hz, H-6), 3.81 (s, 3H, ArOC$_3$H), 3.74 (d, 1H, H-3), 3.63 – 3.61 (m, 2H, H-1'), 3.06 (s, 3H, OCH$_2$OCH$_3$); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ 165.8 (CO), 160.3 (ArC), 134.4 (OCH$_2$CH=CH$_2$), 133.5 (ArCH), 129.9 (ArC), 129.9 (ArCH), 129.6 (ArC), 128.6 (ArCH), 127.8 (ArCH), 116.8 (OCH$_2$CH=CH$_2$), 113.7 (ArCH), 102.4 (OCHO), 97.3 (OCH$_2$O), 93.0 (C-2), 82.1 (C-3), 73.0 (C-4), 71.7 (C-5), 68.1 (C-1), 62.3 (OCH$_2$CH=CH$_2$), 60.9 (C-6), 55.8 (OCH$_2$OCH$_3$), 55.4 (ArOCH$_3$); HRMS-ESI: calcd for C$_{26}$H$_{30}$O$_9$Na: 509.1788; Found: 509.1788 (M+Na$^+$).
4.22 – 4.13 (m, 3H, H-4 and CH₂), 3.85 (dd, 2H, J = 5.9, 1.4 Hz, H-6), 3.78 (s, 3H, ArOCH₃), 3.39 (s, 3H, OCH₂OCH₃), 0.90 (s, 9H, C(CH₃)₃), 0.86 (s, 9H, C(CH₃)₃), 0.08 (s, 3H, SiCH₃), 0.03 (m, 9H, 3SiCH₃); ¹³C NMR (CDCl₃, 125 MHz): δ 206.3 (C=O), 165.8 (C=O), 159.4 (ArC), 133.2 (ArCH), 130.0 (ArCH), 129.9 (ArC), 129.6 (ArCH), 128.5 (ArCH), 113.8 (ArCH), 98.2 (CH₂), 77.5 (C-4), 76.3 (C-3), 73.1 (CH₂), 72.9 (CH₂), 60.5 (C-5), 56.4 (CH₂), 55.4 (ArOCH₃), 25.9 (C(CH₃)₃), 18.2 (C(CH₃)₃), -4.8 (SiCH₃), -4.8 (SiCH₃), -5.2 (SiCH₃), -5.4 (SiCH₃); HRMS-ESI: calcd for C₃₅H₅₆O₉Si₂Na: 699.3361; Found: 699.3354 (M+Na⁺).

(1S,4R,5R)-4-(Tert-butyldimethylsilyl)oxy)-3-methacryloyl-5-(methoxymethoxy)cyclopent-2-en-1-yl benzoate (7). To a stirred solution of 28 (20.0 mg; 0.045 mmol) in dichloromethane (1.0 mL), MnO₂ (39 mg; 0.45 mmol) was added. The resulting solution was stirred at room temperature for 48 h. The mixture was filtered through celite with dichloromethane (50 mL) and the solvent was removed. The crude was re-dissolved in dichloromethane (1.0 mL), MnO₂ (20 mg; 0.23 mmol) was added and the resulting mixture was stirred at room temperature for additional 6 h. The mixture was filtered through celite with dichloromethane (50 mL) and the solvent was removed. Flash chromatography (EtOAc-cyclohexane 1:10) gave 7 as a yellow oil (18.7 mg; 93%); ¹H NMR (CDCl₃, 500 MHz): δ 8.12 – 8.06 (m, 2H, ArCH), 7.61 – 7.56 (m, 1H, ArCH), 7.46 (t, 2H, J = 7.8 Hz, ArCH), 6.16 (d, 1H, J₁,₂ = 1.9 Hz, H-2), 6.00 (s, 1H, C(Me)=CH₂), 5.90 (s, 1H, C(Me)=CH₂), 5.71 (dd, 1H, J₁,₅ = 4.4 Hz, H-1), 5.02 (d, 1H, J₄,₅ = 4.0 Hz, H-4), 4.77 (q, 2H, J = 6.8 Hz, OCH₂O), 4.29 (appt, 1H, J₁,₄ = J₁,₅ = 4.2 Hz, H-5), 3.33 (s, 3H, OCH₃), 1.93 (s, 3H, C(Me)=CH₂), 0.85 (s, 9H, C(CH₃)₃), 0.15 (s, 3H, SiCH₃), 0.07 (s, 3H, SiCH₃); ¹³C NMR (CDCl₃, 125 MHz): δ 194.5 (C=O), 166.1 (C=O), 146.4 (C), 144.5 (C), 133.4 (ArCH), 133.2 (C-2), 130.0 (C), 129.9 (ArCH), 128.8 (C(Me)=CH₂), 128.6 (ArCH), 97.2 (OCH₂O), 90.6 (C-5), 79.9 (C-1), 78.7 (C-4), 56.1 (OCH₃), 25.8 (C(CH₃)₃), 18.1 (C(Me)=CH₂), 17.4 (C(CH₃)₃), -4.6 (SiCH₃), -5.0 (SiCH₃); HRMS-ESI: calcd for C₂₄H₃₄O₆SiNa: 469.2022; Found: 469.2015.

Cell Culture. A549 (non-small cell lung cancer, human), HT29 (colorectal adenocarcinoma, human), SW620 (colorectal, Dukes’ type C, human) and Ls174t (colorectal, Dukes’ type B, human), HeLa (cervix adenocarcinoma, human) cells were grown in 75 cm² culture flasks (Corning® Flask) as adherent monolayer cultures in Dulbecco’s Modified Eagle Medium (DMEM) (GIBCO, ref. 41966-029). Culture mediums were supplemented with 10% heat-inactivated fetal bovine serum, and with Penicillin/Streptomycin (Gibco, 15140-122). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂ and 95% air.
**Inhibition of Cell Viability Assay.** Drug effects on exponentially growing tumour cells were determined using XTT assay as described previously. A549, HT29, SW620, Ls174t, HeLa cells were seeded at a density of 3000 cells/well in 96-well plates and incubated for 24h. Thereafter, cancer cells were exposed to drugs at different concentrations during 72h. At 72h, 50 μL of XTT/ECR 1:50 solution was added and incubated for a further 4 h at 37 °C. Absorbance measured at 475 nm was converted to percentages. UV-vis absorbance was measured at 475 nM using a microplate reader. Experiments were performed in triplicated for each drug concentration and carried out independently at least three times. The interpolation analysis was done using dose-dependent inhibition pattern (log3d vs. normalized response {Variable slope}) with Prism version 5.00 software (GraphPad Software, USA).

**Drug transporter inhibition assay.** Cells are seeded in a 96-well culture plate typically at 20,000 cells/well and are used on days 2 or 3 post-seeding. On the day of assay the test compound is prepared in assay buffer (HBSS-HEPES, pH 7.4), added to the cell plate, and pre-incubated at 37 °C for 15 min. Subsequently substrate is added to the plate followed by 20-min incubation at 37 °C. The plate is then washed with cold assay buffer followed by fluorescence reading for assays with fluorogenic substrates. Cell lines: MDR1-MDCKII (Transporter: P-gp, substrate: Calcein AM, positive control Verapamil IC50 = 29 µM); MRP2-HEK (Transporter: MRP2, substrate CDCF, positive control MK571 IC50 = 14 µM); -HEK; MRP3-HEK (Transporter: MRP2, substrate CDCF, positive control MK571 IC50 = 12 µM).

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