ESK242 and ESK246, monoterpene glycosides from *Pittosporum venulosum*, target LAT3 amino acid transport and prostate cancer cell growth

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Supporting Information

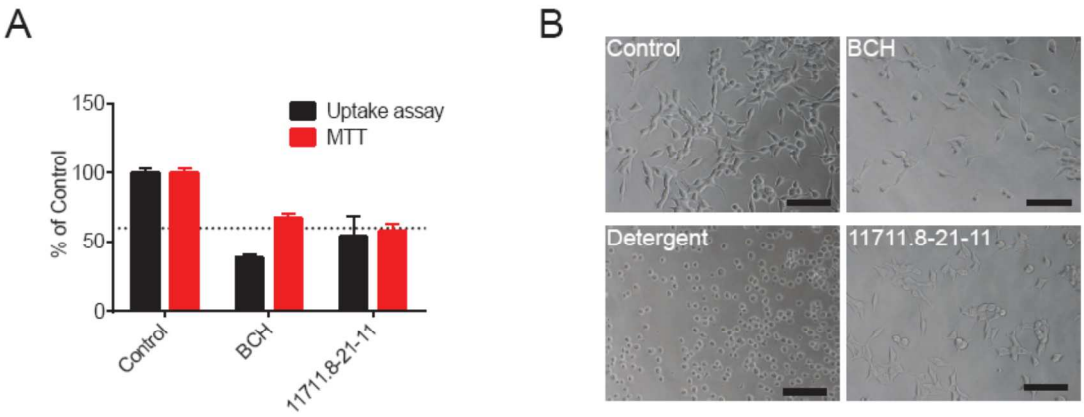
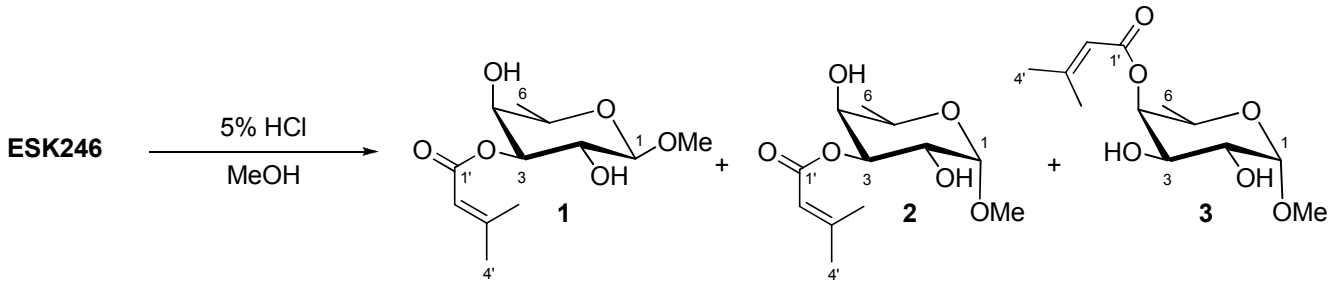


Figure S1. (A) Verification of candidate fraction 11711.8-21-11 by MTT assay and leucine uptake assay. (B) Phase contrast images of LNCaP cells treated with DMSO, BCH (10 mM), detergent (0.1% SDS) and fraction 11711.8-21-11 for 48 h. Scale bar is 100 μm.

Table S1. NMR Spectroscopic data (600 MHz, C_6H_6) for ESK246 and ESK242.

*interchangeable signals

	ESK246		**ESK242**	
	δ_C (mult)	**δ_H (mult *J* in Hz)**	**δ_C (mult)**	**δ_H (mult *J* in Hz)**
1	133.6 (C)		133.6 (C)	
2	121.4 (CH)	5.45 (m)	121.3 (CH)	5.46 (m)
3	26.96 (CH_2)	a 2.12 (m)	27.0 (CH_2)	a 2.12 (m)
		b 1.88 (m)		b 1.90 (m)
4	44.4 (CH)	1.61 (m)	44.4 (CH)	1.61 (m)
5	24.0 (CH_2)	a 1.94 (m)	24.0 (CH_2)	a 1.88 (m)
		b 1.31 (m)		b 1.66 (m)
6	31.3 (CH_2)	a 1.97 (m)	31.3 (CH_2)	a 1.98 (m)
		b 1.88 (m)		b 1.92 (m)
7	23.53* (CH_3)	1.66 (br s)	23.5 (CH_3)	1.66 (br s)
8	79.4 (C)		79.5 (C)	
9	23.52* (CH_3)	1.10 (s)	23.8 (CH_3)	1.09 (s)
10	23.9 (CH_3)	1.19 (s)	23.6 (CH_3)	1.18 (s)
1'	95.9 (CH)	4.47 (d, 7.8)	95.8 (CH)	4.44 (d, 7.8)
2'	70.0 (CH)	5.66 (dd, 10.3, 7.8)	69.85 (CH)	5.60 (dd, 10.4, 7.8)
3'	73.8 (CH)	5.16 (dd, 10.4, 3.2)	74.4 (CH)	5.09 (dd, 10.4, 3.2)
4'	70.4 (CH)	3.72 (br d, 3.2)	70.2 (CH)	3.68 (br dd, 3.2)
5'	70.3 (CH)	3.10 (br q, 6.3)	70.1 (CH)	3.03 (br q, 6.2)
6'	16.6 (CH_3)	1.17 (d, 6.3)	16.5 (CH_3)	1.10 (d, 6.2)
1"	168.9 (C)		168.7 (C)	
2"	20.7 (CH_3)	1.87 (s)	20.6 (CH_3)	1.81 (s)
1"'	165.8 (C)		167.1 (C)	
2"'	116.4 (CH)	5.77 (m)	128.3 (C)	
3"'	157.7 (C)		139.3 (CH)	5.79 (m)
4"'	27.02 (CH_3)	2.14 (d, 1.0)	16.0 (CH_3)	2.05 (m)
5"'	20.2 (CH_3)	1.45 (d, 1.0)	20.8 (CH_3)	1.94 (m)



S1.1 Acid hydrolysis of ESK246

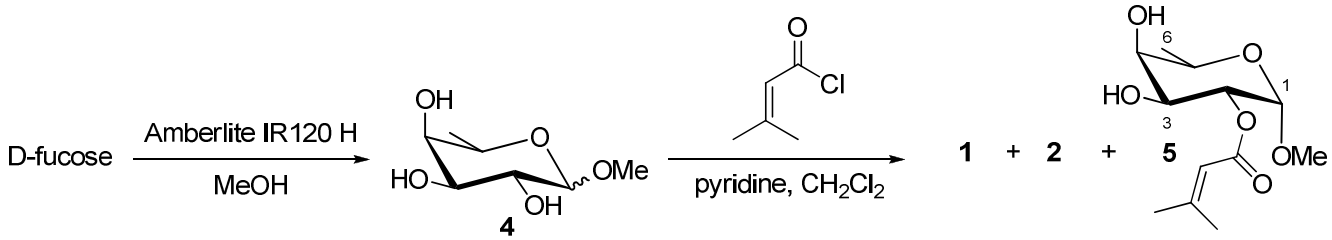
A solution of ESK246 (20.0 mg, 44.7 µM) in MeOH (2.0 mL) was treated with 5% HCl (100 µL) and the reaction was kept stirring at 60ºC for 24 hrs. After hydrolysis the solution was evaporated and subjected to semi-preparative HPLC. Isocratic HPLC conditions of 90% H_2O /10% MeOH were initially employed for 10 min, then a linear gradient to MeOH was run over 40 min, followed by isocratic conditions of MeOH for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min, and then analyzed by (+)-LR-ESIMS and NMR. Fraction 6 yielded **1** (0.7 mg), fraction 9 yielded **2** (0.6 mg) and fraction 18 yielded **3** (0.5 mg).

Methyl-3-*O*-senecioyl-*β*-fucopyranoside (1): clear oil; $[\alpha]_D$ = +70 (*c* 0.05, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) 5.72 (m, 1H, H-2'), 4.57 (dd, *J* = 10.0, 3.5 Hz, 1H, H-3), 4.11 (d, *J* = 7.8 Hz, 1H, H-1), 3.61 (br q, *J* = 6.5 Hz, 1H, H-5), 3.57 (dd, *J* = 4.0, 3.2 Hz, 1H, H-4), 3.49 (dd, *J* = 10.0, 7.8 Hz, 1H, H-2) 3.37 (s, 3H, 1-OMe), 2.11 (d, *J* = 1.0 Hz, 3H, H-4'), 1.88 (d, *J* = 1.0 Hz, 3H, H-5'), 1.12 (d, *J* = 6.5 Hz, 3H, H-6); ^{13}C NMR (150 MHz, DMSO-d_6) 165.3 (C-1'), 156.4 (C-3'), 116.0 (C-2'), 104.0 (C-1), 75.3 (C-3), 69.6 (C-5), 68.4 (C-4), 67.1 (C-2), 55.7 (1-OMe), 26.7 (C-5'), 19.8 (C-4'), 16.3 (C-6); LRESIMS *m/z* $[M+Na]^+$ 283.1.

Methyl-3-*O*-senecioyl-*α*-fucopyranoside (2): clear oil; $[\alpha]_D$ = +120 (*c* 0.045, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) 5.72 (m, 1H, H-2'), 4.82 (dd, *J* = 10.8, 3.4 Hz, 1H, H-3), 4.55 (d, *J* = 3.7 Hz, 1H, H-1), 3.83 (dd, *J* = 10.8, 3.7 Hz, 1H, H-2), 3.80 (br q, *J* = 6.5 Hz, 1H, H-5), 3.64 (br s, 1H, H-4), 3.28 (s, 3H, 1-OMe), 2.10 (d, *J* = 1.0 Hz, 3H, H-4'), 1.88 (d, *J* = 1.0 Hz, 3H, H-5'), 1.09 (d, *J* = 6.5 Hz, 3H, H-6); ^{13}C NMR (150 MHz, DMSO-d_6) 165.3 (C-1'), 155.7 (C-3'), 116.1 (C-2'), 100.0 (C-1), 72.4 (C-3), 68.9 (C-4), 65.4 (C-2), 65.0 (C-5), 54.5 (1-OMe), 26.7 (C-5'), 19.7 (C-4'), 16.2 (C-6); LRESIMS *m/z* $[M+Na]^+$ 283.1.

Methyl-4-*O*-senecioyl-*α*-fucopyranoside (3): clear oil; $[\alpha]_D$ = +77.5 (*c* 0.04, MeOH); ^{1}H NMR (600 MHz, DMSO-d_6) 5.73 (m, 1H, H-9), 5.04 (dd, *J* = 3.6, 1.3 Hz, 1H, H-4), 4.84 (d, *J* = 5.2 Hz, 1H, 3-

OH), 4.65 (d, *J* = 6.9 Hz, 1H, 2-OH), 4.56 (d, *J* = 3.8 Hz, 1H, H-1), 3.93 (m, 1H, H-5), 3.73 (ddd, *J* = 10.7, 5.2, 3.6 Hz, 1H, H-3), 3.54 (ddd, *J* = 10.7, 6.9, 3.8 Hz, 1H, H-2), 3.27 (s, 3H, 1-OMe), 2.11 (d, *J* = 1.0 Hz, 3H, H-4'), 1.89 (d, *J* = 1.0 Hz, 3H, H-5'), 0.95 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C NMR (150 MHz, DMSO-d_6) 165.4 (C-1'), 155.9 (C-3'), 115.8 (C-2'), 100.1 (C-1), 72.9 (C-4), 68.3 (C-2), 67.3 (C-3), 64.2 (C-5), 54.6 (1-OMe), 26.7 (C-5'), 19.8 (C-4'), 16.1 (C-6); LRESIMS *m/z* $[M+Na]^+$ 283.1.



***S1.2 Synthesis of* methyl-3-*O*-senecioyl-*α*-D-fucopyranoside**

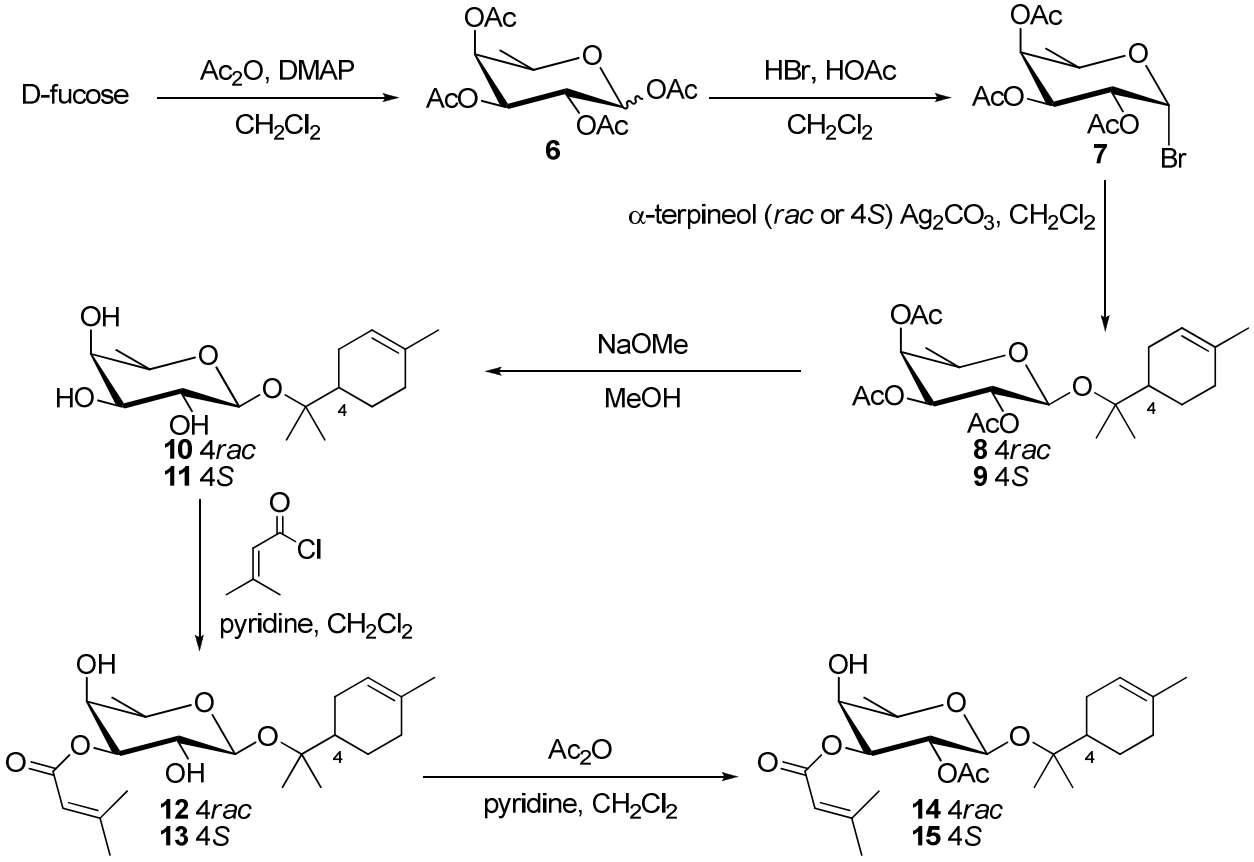
A stirring mixture of D-fucose (300 mg, 1.8 mmol) and methanol-washed Amberlite® IR120 H resin (300 mg) in anhydrous methanol (4 mL) was heated to reflux for 16 h. The cooled reaction mixture was decanted from the resin, and 2 drops of triethylamine were added to the solution. The solution was filtered through celite and evaporated to yield **4** a colourless solid (290 mg), which was used without further purification. The crude solid was dissolved in pyridine/CH_2Cl_2 1:4 (5 mL) and cooled to 0°C. To the stirring solution was added freshly prepared 3,3-dimethylacryloyl chloride[1] (90 µL, 0.8 mmol) dropwise. The reaction mixture was allowed to warm to room temperature (RT) and stirred for 16 h, after which time the mixture was evaporated and the residue was purified by column chromatography on silica gel (hexane 100 % to 5 : 4 : 1 hexane : EtOAc : MeOH) to give an impure colourless oil (123 mg). The residue was dissolved in methanol (2.5 mL), and 0.5 mL of this solution was evaporated and subjected to purification by semipreparative HPLC._Isocratic HPLC conditions of 90% H_2O /10% MeOH were initially employed for 10 min, then a linear gradient to MeOH was run over 40 min, followed by isocratic conditions of MeOH for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min, and then analyzed by (+)-LR-ESIMS and NMR. Fraction 3 yielded **1** (3.1 mg), fractions 5 and 6 yielded **2** (6.2 mg) and fractions 12-16 yielded **5** (5.7 mg).

Methyl-3-*O*-senecioyl-*β*-D-fucopyranoside (1): clear oil; $[\alpha]_D$ = +54 (*c* 0.05, MeOH); ^{1}H NMR and ^{13}C NMR data identical to that reported for the hydrolysis-sourced product; HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{20}O_6Na$, 283.11521; found, 283.11556.

Methyl-3-*O*-senecioyl-*α*-D-fucopyranoside (2): clear oil; $[\alpha]_D$ = +129 (*c* 0.045, MeOH); ^{1}H NMR and ^{13}C NMR data identical to that reported for the hydrolysis-sourced product; HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{20}O_6Na$, 283.11521; found, 283.11545.

Methyl-2-*O*-senecioyl-*α*-D-fucopyranoside (5): clear oil; $[\alpha]_D$ = +60 (*c* 0.05, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) 5.70 (m, 1H, H-9), 4.84 (dd, *J* = 10.5, 3.7 Hz, 1H, H-2), 4.73 (d, *J* = 5.9 Hz, 1H, 3-OH), 4.71 (d, *J* = 4.7 Hz, 1H, 4-OH), 4.67 (d, *J* = 3.7 Hz, 1H, H-1), 3.78 (br q, *J* = 6.5 Hz, 1H, H-5), 3.76 (m, 1H, H-3), 3.56 (m, 1H, H-4), 3.22 (s, 3H, 1-OMe), 2.11 (d, *J* = 1.0 Hz, 3H, H-4'), 1.88 (d, *J* = 1.0 Hz, 3H, H-5'), 1.12 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C NMR (125 MHz, DMSO-d_6) 165.5 (C-1'), 156.8 (C-3'), 115.6 (C-2'), 96.9 (C-1), 71.5 (C-4), 70.1 (C-2), 66.8 (C-3), 65.7 (C-5), 54.4 (1-OMe), 27.0 (C-5'), 19.8 (C-4'), 16.2 (C-6); HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{20}O_6Na$, 283.11521; found, 283.11542.

S1.2 Synthesis of ESK246 diastereomers



1,2,3,4-Tetra-*O*-acetyl-D-fucopyranose (6): D-Fucose (2.00 g, 12.18 mmol) was added to a solution of *N,N*-dimethylaminopyridine (DMAP) (149 mg, 1.22 mmol) in Ac_2O (9.21 mL, 97.44 mmol) at 0°C. The reaction mixture was warmed to RT and stirred for 18 h. The mixture was poured over crushed ice, and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with H_2O (3 × 60 mL), $NaHCO_3$

(2 × 60 mL), and brine (2 × 60 mL), dried (Na_2SO_4) and evaporated to give the title compound (3.91 g, 97%, mixture of anomers α:β, 2:3 determined by ^{1}H NMR) as a colourless oil without further purification. Spectroscopic data was consistent with literature.[2] ^{1}H NMR (500 MHz, $CDCl_3$) 6.30 (d, *J* = 3.0 Hz, 1H), 5.65 (d, *J* = 8.5 Hz, 1H), 5.30-5.23 (m, 5H), 5.05 (dd, *J* = 10.5, 3.5 Hz, 1H), 4.24 (q, *J* = 6.5 Hz, 1H), 3.93 (q, *J* = 6.5 Hz, 1H), 2.15-1.95 (series of s, 3H), 1.19 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 170.48, 170.46, 170.11, 169.94, 169.90, 169.4, 169.1, 166.4, 92.2, 89.9, 71.3, 70.6, 70.3, 70.0, 68.0, 67.9, 67.3, 66.5, 22.1, 20.9, 20.8, 20.63, 20.60, 20.57, 20.53, 15.92, 15.91.

1,2,3,4-Tetra-*O*-acetyl-D-fucopyranosyl bromide (7): A solution of **6** (3.92 g, 11.81 mmol) in CH_2Cl_2 (30 mL) was treated with HBr/HOAc (8.75 mL, 33%) at room temperature, and the resulting mixture was stirred for 3 h. The reaction mixture was poured over crushed ice and solid $NaHCO_3$ was added until effervescence stopped. The mixture was diluted with CH_2Cl_2 (50 mL) and extracted with H_2O (3 × 60 mL). The organic layer was dried (Na_2SO_4), filtered and the solvents evaporated to give the title compound (3.43 g, 82%) as a yellow oil without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) 6.68 (d, *J* = 4.0, 1H), 5.40 (dd, *J* = 10.5, 3.0 Hz, 1H), 5.35 (d, *J* = 3.0 Hz, 1H), 5.01 (dd, *J* = 10.5, 4.0 Hz, 1H), 4.39 (q, *J* = 6.5 Hz, 1H), 2.16-1.99 (series of s, 3H), 1.20 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 170.4, 170.2, 169.9, 89.4, 70.2, 69.9, 68.6, 68.0, 20.9, 20.7, 20.6, 15.7.

(4*rac*)-α-terpineol-8-*O*-β-D-(2',3',4'-tri-acetyl) fucopyranoside (8): Fucopyranosyl bromide (2.01 g, 5.69 mmol) was dissolved in dry CH_2Cl_2 (15 mL) and stirred with 3Å molecular sieves under N_2 for 3 h. A solution of α-terpineol (0.96 g, 6.25 mmol) in dry CH_2Cl_2 (25 mL) was added under N_2 to a foil-covered flask containing a stirring mixture of silver carbonate (Ag_2CO_3) (1.88 g, 6.82 mmol) and 3Å molecular sieves. After 3 h, the fucopyranosyl bromide solution was transferred under N_2 to the foil covered flask containing α-terpineol and Ag_2CO_3. The resulting mixture was stirred at RT for 48 h and then filtered through Celite. The solvents were evaporated and the residue purified by column chromatography on silica gel (hexane 100 % → 3:7 EtOAc/hexane) to give the title compound (1.18 g, 49%) as a clear syrup. R_f 0.63 (3:7 EtOAc/hexane). ^{1}H NMR (500 MHz, $CDCl_3$) 5.34 (br s, 2H), 5.19 (m, 2H), 5.14 (m, 2H), 5.01 (m, 2H), 4.60 (d, *J* = 7.5 Hz, 2H), 3.75 (m, 2H), 2.03 – 1.90 (m, 16H), 1.81 – 1.64 (m, 4H), 1.61 (br s, 6H), 1.57 (m, 2H), 1.22 – 1.14 (m, 22H); ^{13}C NMR (125 MHz, $CDCl_3$) 170.9, 170.4, 169.4, 134.4, 133.8, 120.9, 120.5, 95.54, 95.49, 80.4, 80.3, 71.80, 71.75, 70.59, 70.58, 69.38, 68.95, 68.91, 43.90, 43.85, 31.2, 31.0, 27.1, 26.6, 25.2, 24.3, 23.9, 23.53, 23.46, 23.42, 23.1, 21.9, 20.95,

20.93, 20.86, 20.75, 16.45 16.42. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{22}H_{34}O_8Na$, 449.214589; found, 449.214621.

(4*S*)-α-Terpineol-8-*O*-*β*-D-(2',3',4'-tri-acetyl) fucopyranoside (9): An equivalent procedure using (4*S*)-α-terpineol yielded **9** (0.94 g, 39%) as a clear syrup. $[\alpha]_D$ = -49.5 (*c* 0.1, MeOH); R_f 0.63 (3:7 EtOAc/hexane). ^{1}H NMR (500 MHz, $CDCl_3$) 5.31 (m, 1H), 5.18 (d, *J* = 3.5, 1H), 5.13 (dd, *J* = 10.5, 7.5, 1H), 5.00 (dd, *J* = 10.5, 3.5, 1H), 4.59 (d, *J* = 7.5, 1H), 3.74 (q, *J* = 6.5, 1H), 2.13 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.73 (m, 2H), 1.60 (br s, 3H), 1.56 (m, 1H), 1.25 – 1.12 (m, 2H), 1.17-1.15 (m, 6H), 1.08 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 170.9, 170.3, 169.3, 134.3, 120.4, 95.5, 80.4, 71.7, 70.5, 69.3, 68.9, 43.8, 30.9, 27.0, 25.1, 23.5, 23.4, 21.9, 20.9, 20.8, 20.7, 16.4. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{22}H_{34}O_8Na$, 449.214589; found, 449.214560.

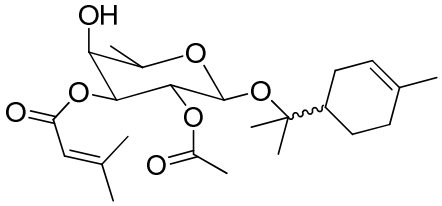
(4*rac*)-α-terpineol-8-*O*-*β*-D-fucopyranoside (10): A solution of **8** (1.04 g, 2.44 mmol) in dry MeOH (15 mL) was treated with sodium methoxide (13 mg, 0.24 mmol), the resulting mixture was stirred under N_2 at room temperature for 3 h. The solution was evaporated and the residue purified by flash column chromatography on silica gel (5:4:1 hexane/EtOAc/MeOH) to give the title compound (0.62 g, 85%) as a clear syrup. R_f 0.46 (5:4:1 hexane/EtOAc/MeOH). ^{1}H NMR (500 MHz, CD_3OD) 5.44 (br s, 2H), 4.47 (d, *J* = 7.5 Hz, 2H), 3.68 (m, 4H), 3.54 (dd, *J* = 9.5, 3.5 Hz, 2H), 3.49 (dd, *J* = 9.5, 7.5 Hz, 2H), 2.15 – 1.86 (m, 10H), 1.85 – 1.72 (m, 4H), 1.70 (s, 6H), 1.25 – 1.15 (m, 18H). ^{13}C NMR (125 MHz, CD_3OD) 134.79, 134.69, 121.97, 121.92, 98.76, 98.71, 80.7, 80.6, 75.4, 75.3, 73.1, 72.5, 71.4, 45.1, 45.0, 32.1, 28.2, 27.9, 25.1, 25.0, 24.2, 23.8, 23.57, 23.55, 22.9, 17.1, 16.9. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{28}O_5Na$, 323.182895; found, 323.183845.

(4*S*)-α-terpineol-8-*O*-*β*-D-fucopyranoside (11): Compound **9** was subjected to an equivalent procedure to give the title compound (0.69 g, 95%) as a clear syrup. $[\alpha]_D$ = -19.6 (*c* 0.1, MeOH); R_f 0.46 (5:4:1 hexane/EtOAc/MeOH). ^{1}H NMR (500 MHz, CD_3OD) 5.36 (m, 1H), 4.40 (d, *J* = 7.5 Hz, 1H), 3.60 (m, 2H), 3.47 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.42 (dd, *J* = 10.0, 7.5 Hz, 1H), 2.11 – 1.88 (m, 5H), 1.79 (m, 1H), 1.67 (m, 1H) 1.63 (s, 3H), 1.24 (d, *J* = 6.5 Hz, 3H), 1.21 (s, 3H), 1.17 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD) 134.7, 121.9, 98.7, 80.7, 75.3, 73.0, 72.5, 71.3, 45.0, 32.1, 28.1, 25.1, 25.0, 23.5, 22.9, 17.0. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{28}O_5Na$, 323.182895; found, 323.182322.

(4*rac*)-α-terpineol 8-*O*-*β*-D-(3□-senecioyl)-fucopyranoside (12): A stirring solution of **10** (0.40 g, 1.33 mmol) in pyridine/CH_2Cl_2 1:4 (10 mL) was treated with freshly prepared 3,3-dimethylacryloyl

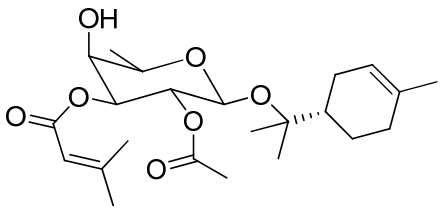
chloride[1] (0.077 mL, 0.66 mmol) at 0°C. The reaction was allowed to reach RT and stirred for 18 h. The solution was evaporated and the residue purified by column chromatography on silica gel (hexane 100% → 1:1 hexane/EtOAc) to give the title compound (0.27 g, 53%) as a clear syrup. R_f 0.49 (1:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) 5.80 (m, 2H), 5.36 (br s, 2H), 4.89 (dd, *J* = 9.5, 3.0 Hz, 2H), 4.49 (d, *J* = 7.5 Hz, 2H), 3.82 (d, *J* = 3.0 Hz, 2H), 3.73 (dd, *J* = 9.5, 7.5 Hz, 2H), 3.68 (q, *J* = 6.5 Hz, 2H), 2.18 (s, 6H), 2.12 – 1.89 (m, 16H), 1.87 – 1.74 (s, 2H), 1.79 (m, 2H), 1.69 (m, 2H), 1.64 (s, 6H), 1.31 – 1.21 (m, 24H); ^{13}C NMR (125 MHz, $CDCl_3$) 166.1, 158.5, 134.4, 134.1, 120.7, 120.6, 115.7, 97.59, 97.55, 80.5, 80.3, 74.94, 74.89, 70.51, 70.50, 70.34, 70.33, 69.63, 69.61, 43.88, 43.78, 31.09, 31.04, 27.6, 27.2, 26.9, 25.1, 24.1, 23.95, 23.93, 23.8, 23.45, 23.42, 22.6, 20.5, 16.57, 16.54. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{21}H_{34}O_6Na$, 405.22476; found, 405.224699.

(4*S*)-terpineol 8-*O*-β-D-(3□-senecioyl)-fucopyranoside (13): Compound **11** was subjected to an equivalent procedure to give the title compound (0.29 g, 57 %) as a clear syrup. $[\alpha]_D$ = +25.5 (*c* 0.1, MeOH); R_f 0.49 (1:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) 5.80 (m, 1H), 5.36 (br s, 1H), 4.89 (dd, *J* = 10.5, 3.5 Hz, 1H), 4.49 (d, *J* = 7.5 Hz, 1H), 3.82 (d, *J* = 3.5 Hz, 1H), 3.73 (dd, *J* = 10.5, 7.5 Hz, 1H), 3.68 (q, *J* = 6.5 Hz, 1H), 2.18 (s, 3H), 2.14 – 1.95 (m, 5H), 1.91 (s, 3H), 1.79 (m, 1H), 1.68 (m, 1H), 1.64 (s, 3H), 1.29 (d, *J* = 6.5 Hz, 3H), 1.23 (s, 3H), 1.18 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) 166.1, 158.6, 134.4, 120.6, 115.7, 97.6, 80.6, 74.9, 70.6, 70.4, 69.7, 43.8, 31.1, 27.6, 27.3, 25.1, 23.99, 23.5, 22.7, 20.5, 16.6. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{21}H_{34}O_6Na$, 405.22476; found, 405.225237.



(4*rac*)-α-terpineol-8-*O*-β-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (14): A stirring solution of **12** (0.27 g, 0.7 mmol) in pyridine/CH_2Cl_2 1:4 (5 mL) was treated with acetic anhydride (0.084 mL, 0.89 mmol) at 0°C. The reaction mixture was allowed to warm to RT and stirred for 18 h. The solution was evaporated and the residue purified by flash column chromatography on silica gel (hexane 100% → 7 : 3 hexane : EtOAc) to give the title compound (0.16 g, 53%) as a white oil. R_f 0.48 (3:7 EtOAc/hexane). ^{1}H NMR (600 MHz, C_6D_6) 5.68 (m, 2H), 5.59 (m, 2H), 5.41 (br s, 2H), 5.04 (m, 2H), 4.43 (d, *J* = 6.5 Hz, 1H), 4.42 (d, *J* = 6.5 Hz, 1H), 3.61 (m, 2H), 3.00 (m, 2H), 2.09 (s, 6H), 2.02 – 1.77 (m, 10H), 1.780 (s,

3H), 1.793 (s, 3H), 1.62 (s. 6H), 1.58 (m, 2H), 1.39 (s, 6H), 1.28 (m, 2H), 1.16 (s, 3H), 1.15 (s, 3H), 1.08 (m, 6H), 1.06 (s, 3H), 1.04 (s, 3H). ^{13}C NMR (150 MHz, C_6D_6) 168.9, 165.7, 157.9, 134.0, 133.7, 121.4, 121.1, 116.3, 96.1, 95.9, 79.6, 79.5, 73.73, 73.68, 70.53, 70.51, 70.2, 70.04, 70.02, 44.5, 31.4, 31.3, 27.3, 27.1, 27.0, 25.2, 24.1, 23.99, 23.92, 23.61, 23.58, 22.36, 20.7, 20.3, 16.61, 16.59. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{36}O_7Na$, 447.235325; found, 447.235960.



(4*S*)-terpineol 8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (15): Compound **13** was subjected to an equivalent procedure to give the title compound (0.15 g, 50%) as a white oil. $[\alpha]_D$ = +21 (*c* 0.1, MeOH); R_f 0.48 (3:7 EtOAc/hexane). ^{1}H NMR (600 MHz, C_6D_6) 5.68 (m, 1H), 5.59 (dd, *J* = 8.5, 6.5 Hz, 1H), 5.40 (br s, 1H), 5.09 (dd, *J* = 8.5, 3.0 Hz, 1H), 4.43 (d, *J* = 6.5 Hz, 1H), 3.61 (m, 1H), 3.00 (q, *J* = 5.0 Hz, 1H), 2.09 (s, 3H), 2.11 – 1.78 (m, 5H), 1.79 (s, 3H), 1.62 (s, 3H), 1.61 (m, 1H), 1.39 (s, 3H), 1.26 (m, 1H), 1.16 (s, 3H), 1.08 (d, *J* = 5.0 Hz, 3H), 1.04 (s, 3H). ^{13}C NMR (150 MHz, C_6D_6) 168.9, 165.7, 157.9, 134.0, 121.1, 116.3, 96.1, 79.6, 73.7, 70.5, 70.2, 70.0, 44.5, 31.3, 27.3, 27.0, 25.2, 23.9, 23.6, 22.4, 20.7, 20.3, 16.6. HRESIFTMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{36}O_7Na$, 447.235325; found, 447.235139.

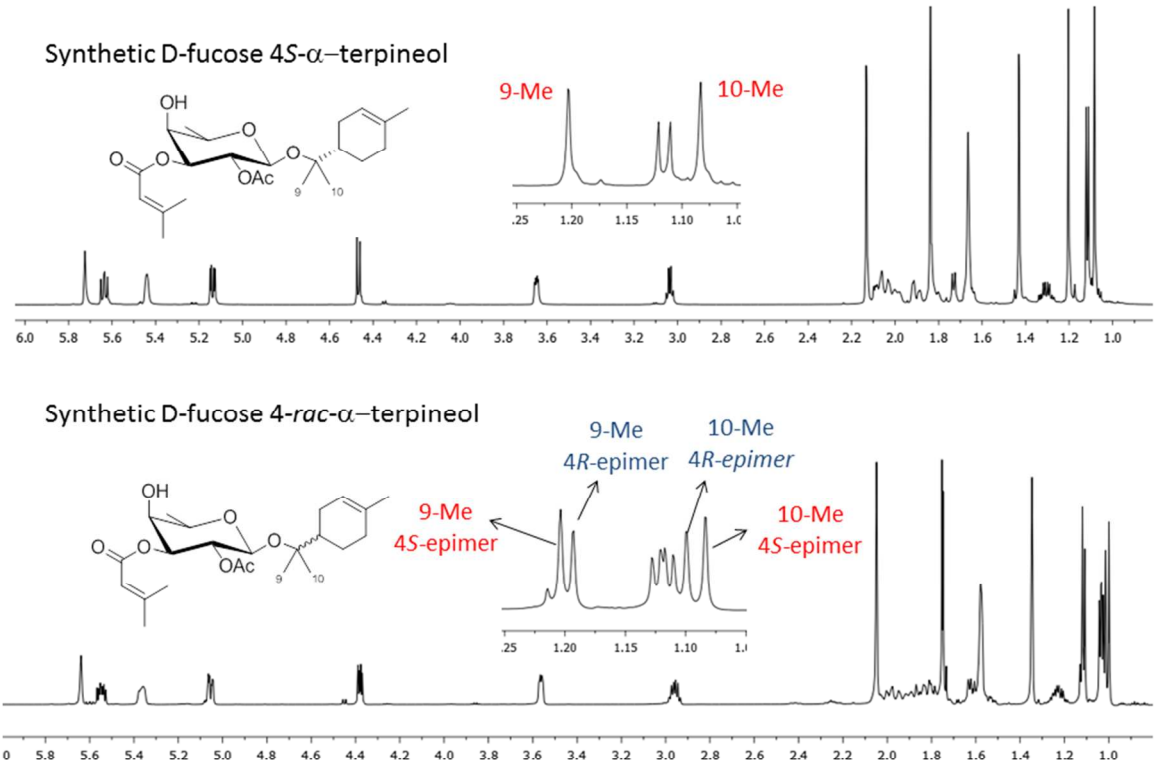


Figure S2. ^{1}H NMR spectrum of (4*S*)-α-terpineol-8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (on top) and the C-4 epimeric mixture of α-terpineol-8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (bottom) in C_6D_6-d_6.

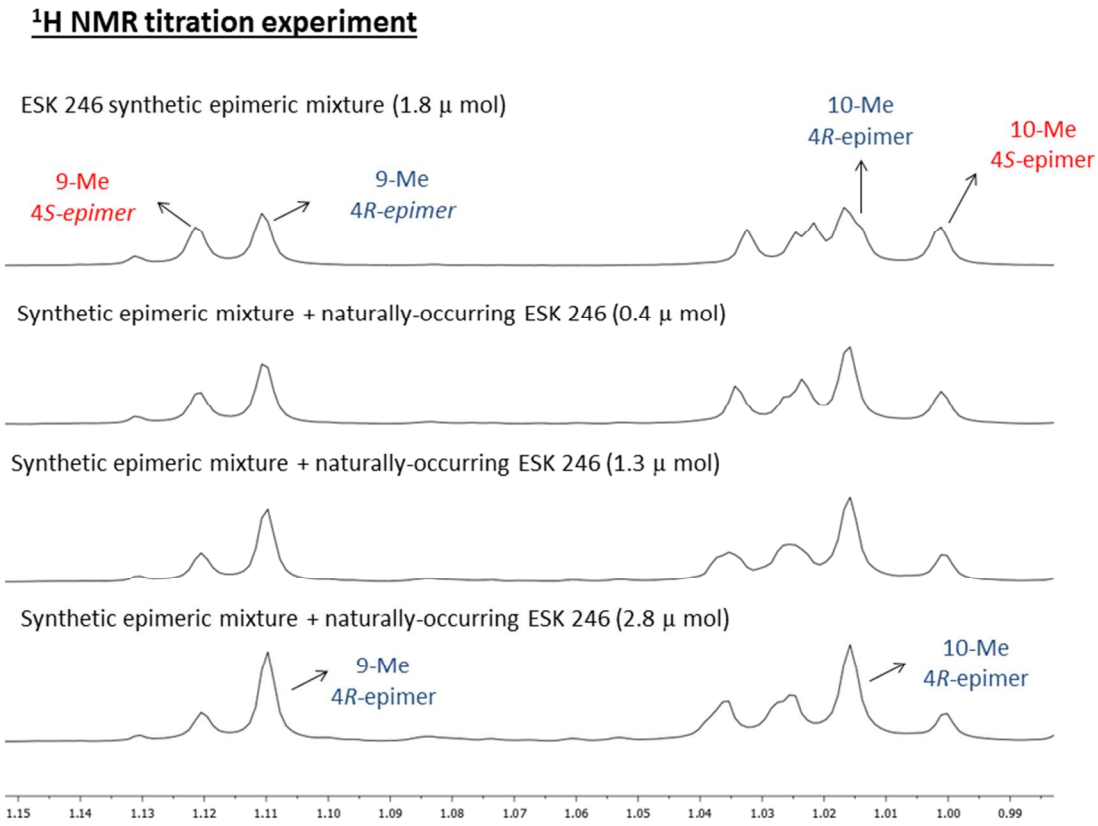


Figure S3. ^{1}H NMR titration experiment. The synthetic C-4 epimeric mixture of α-terpineol-8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside was titrated with the naturally-occurring ESK246. All spectra were recorded in in C_6D_6-d_6.

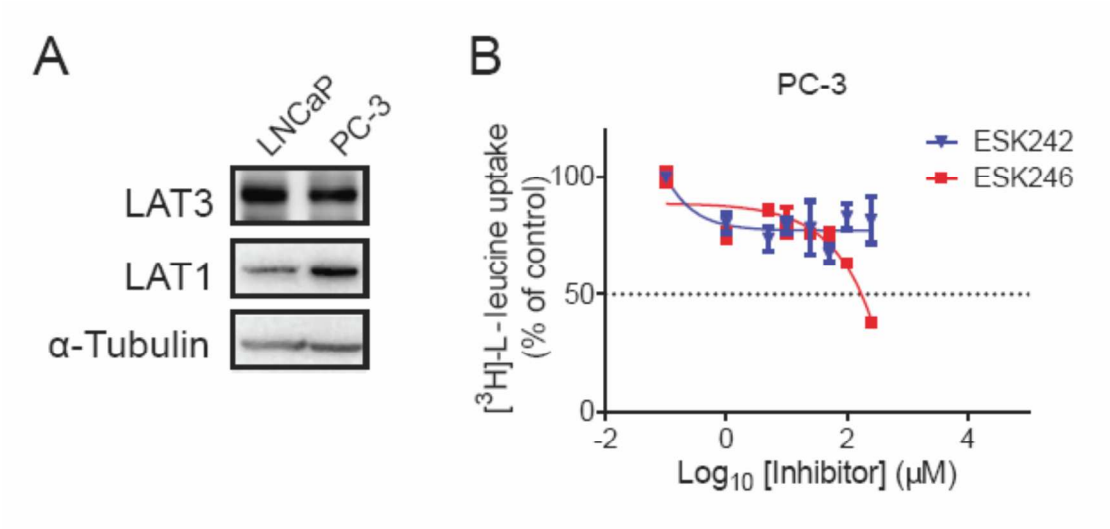


Figure S4. (A) Representative western blots of LAT3 and LAT1 expression in LNCaP and PC-3 cells. α-Tubulin was used as a loading control. (B) Inhibition of [^{3}H]-L-leucine uptake (n=3) in PC-3 cells by ESK242 and ESK246.

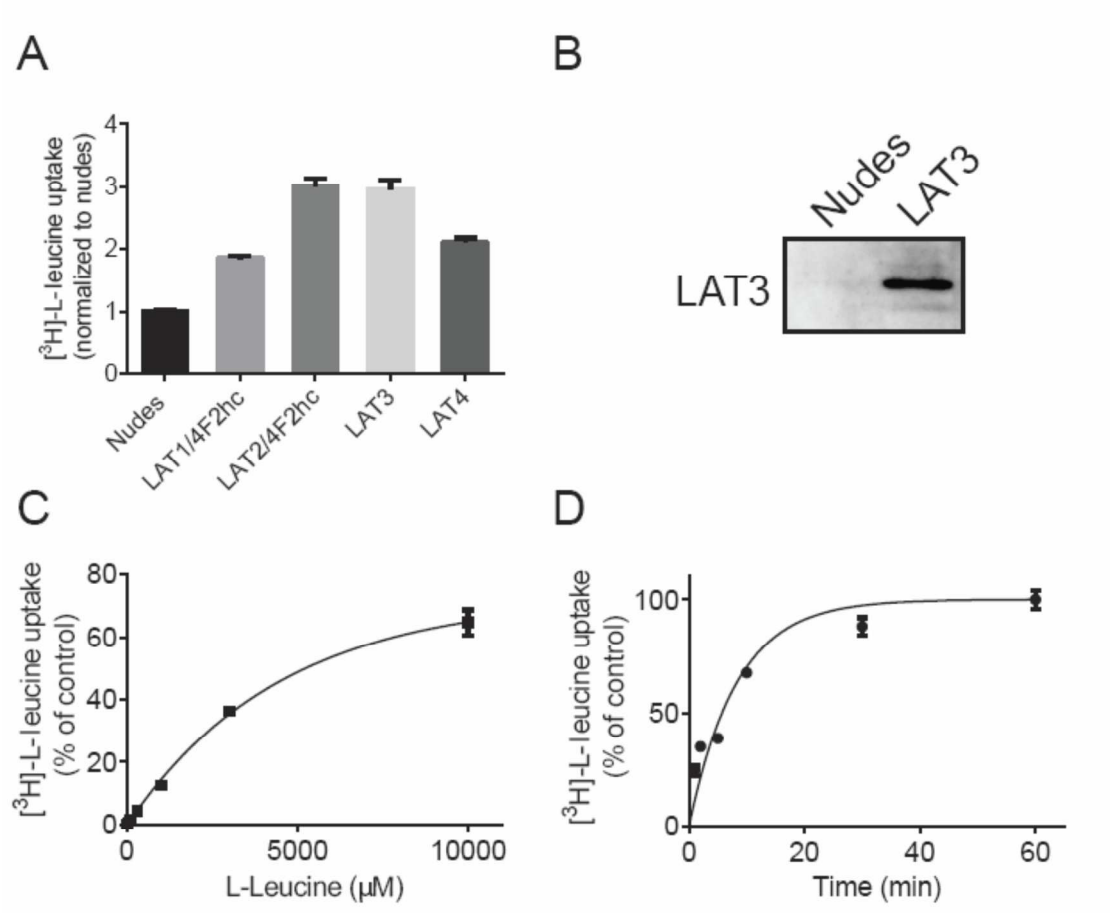


Figure S5. (A) Leucine uptake assay in oocytes expressing LAT1/4F2hc, LAT2/4F2hc, LAT3 or LAT4, compared to nudes (uninjected). 5 oocytes were used for each group. (B) Representative western blots showing expression of human LAT3 on the surface of oocytes. (C) Dose response curve of L-leucine in oocytes. 5 oocytes were used for each time point. (D) Time response curve of L-leucine in the oocytes. 5 oocytes were used for each time point.

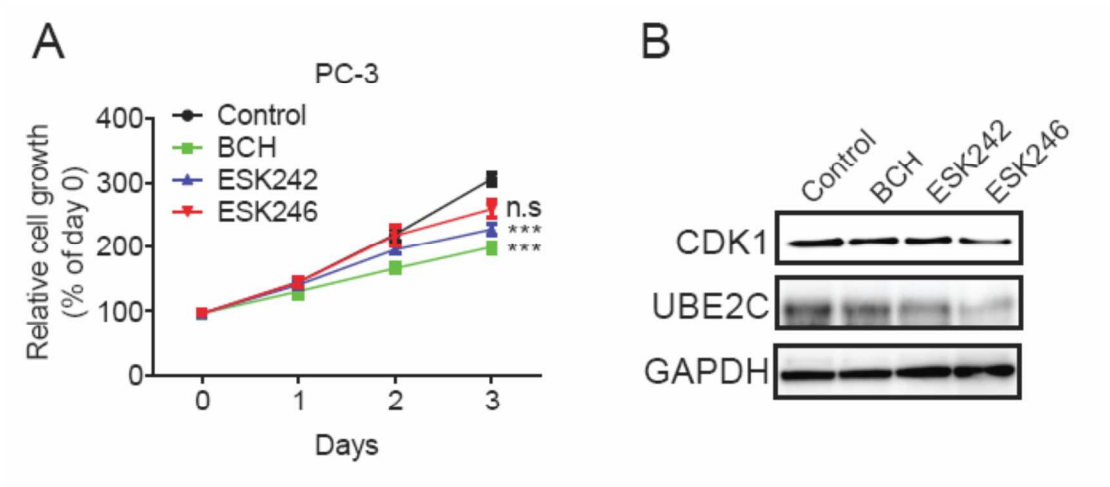


Figure S6. (A) MTT assay (n=3) in PC-3 cells in the absence or presence of BCH, ESK242 and ESK246. Two-way ANOVA test was performed. ***<0.001. (B) Representative western blots (from n=3) of CDK1 and UBE2C expression in LNCaP
cells after BCH (10 mM), ESK242 (50 μM) and ESK246 (50 μM) inhibition. GAPDH was used as the loading control.

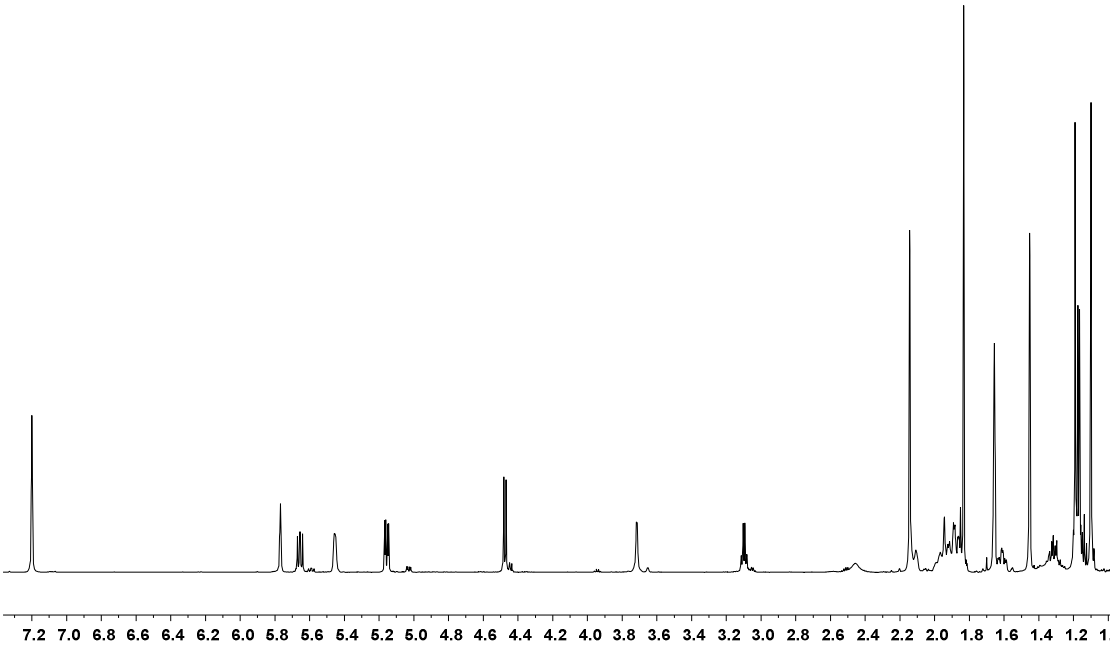


Figure S7. ^{1}H NMR spectrum of ESK246 in C_6D_6-*d_6*.

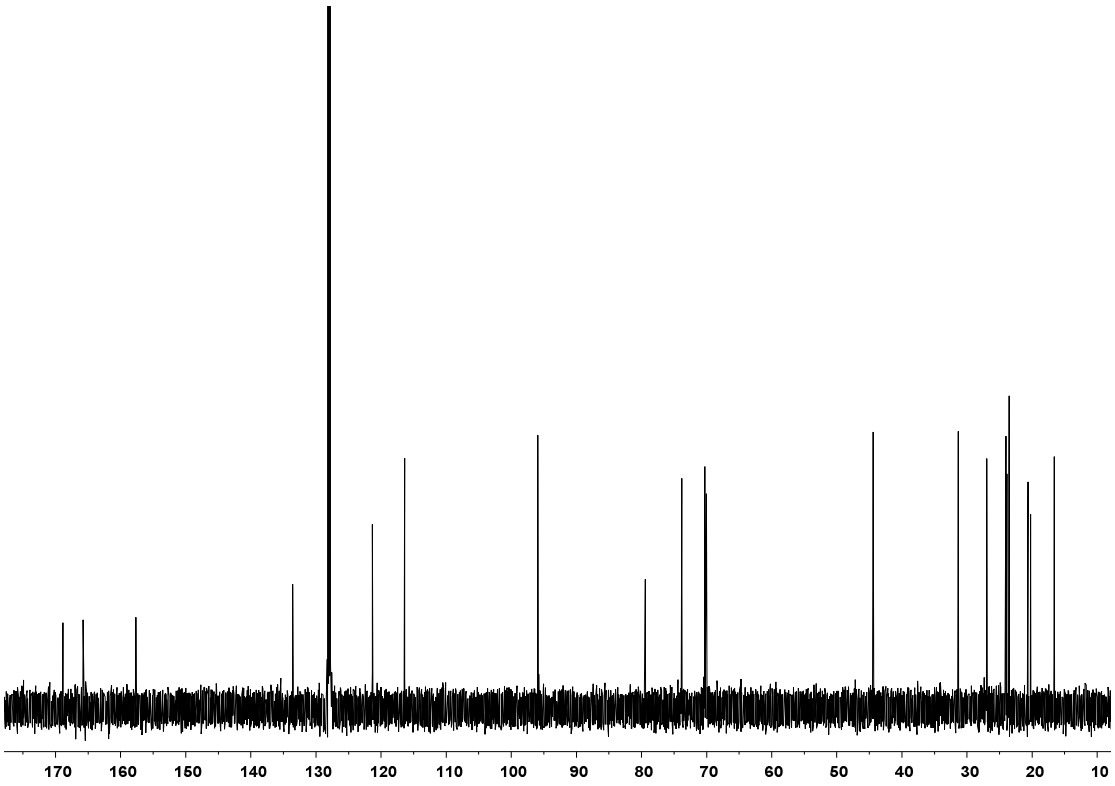


Figure S8. ^{13}C NMR spectrum of ESK246 in C_6D_6-*d*$_6$.

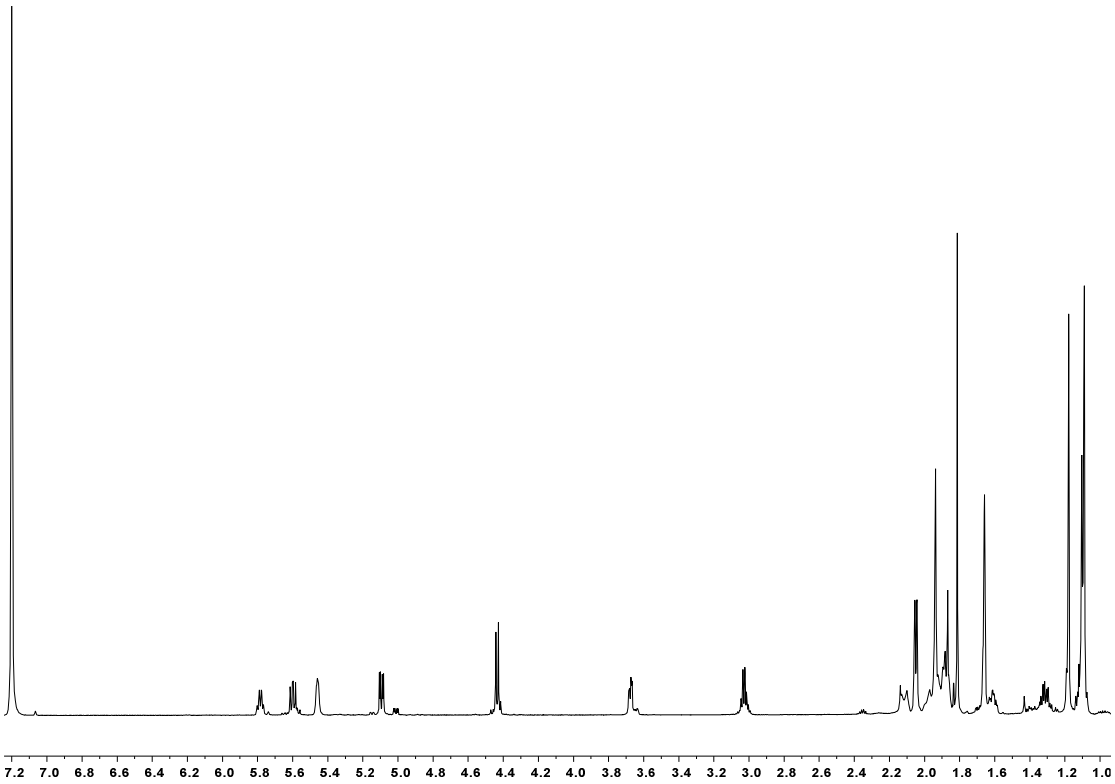


Figure S9. ^{1}H NMR spectrum of ESK242 in C_6D_6-*d*$_6$.

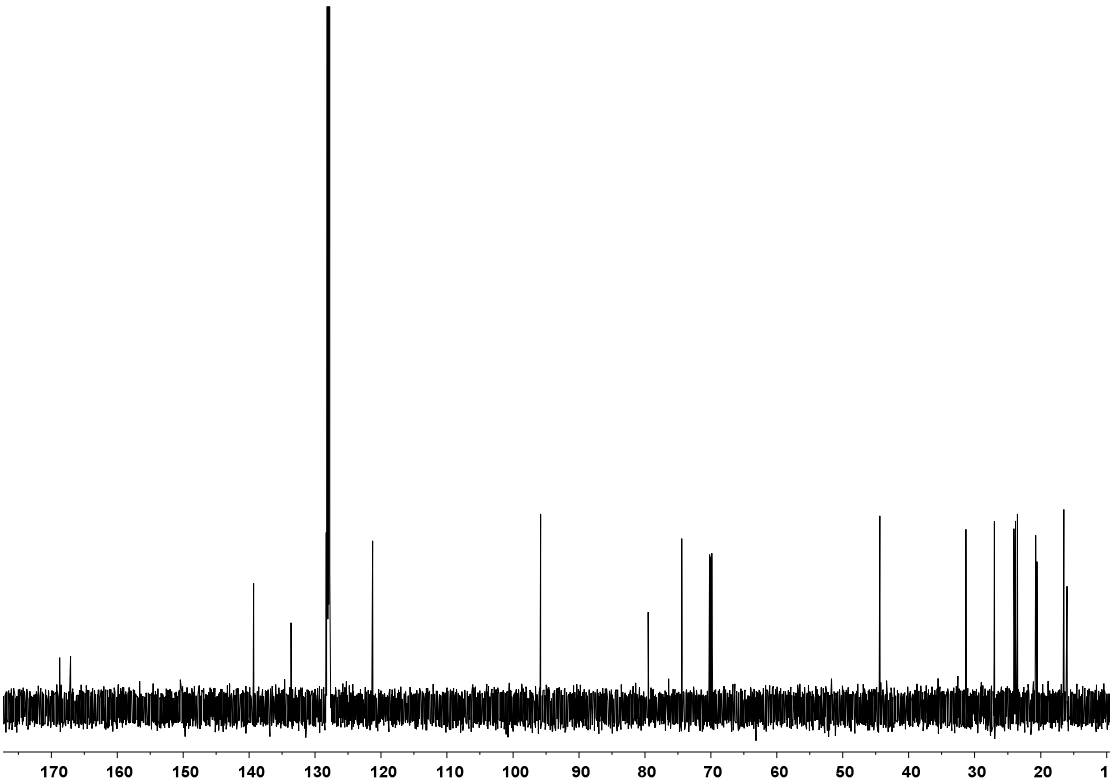


Figure S10. ^{13}C NMR spectrum of ESK242 in C_6D_6-*d*$_6$.

Table S2. Purity of synthetic and natural compounds

Compound	Purity by ^{1}H NMR
(4*rac*)-α-Terpineol-8-*O*-*β*-D-(2',3',4'-tri-acetyl) fucopyranoside (**8**)	>90%
(4*S*)-α-Terpineol-8-*O*-*β*-D-(2',3',4'-tri-acetyl) fucopyranoside (**9**)	>95%
(4*rac*)-α-terpineol-8-*O*-*β*- D -fucopyranoside (**10**)	>95%
(4*S*)-α-terpineol-8-*O*-*β*-D-fucopyranoside (**11**)	>95%
(4*rac*)-α-terpineol 8-*O*-*β*-D-(3□-senecioyl)-fucopyranoside (**12**)	>90%
(4*S*)-α-terpineol 8-*O*-*β*-D-(3□-senecioyl)-fucopyranoside (**13**)	>95%
(4*rac*)-α-terpineol-8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (**14**)	>95%
(4*rac*)-α-terpineol-8-*O*-*β*-D-(2□-acetyl, 3□-senecioyl) fucopyranoside (**15**)	>95%
ESK242 (purified natural product)	>95%
ESK246 (purified natural product)	>95%

References:

1. Bartlett, C. J.; Day, D. P.; Chan, Y.; Allin, S. M.; McKenzie, M. J.; Slawin, A. M. Z.; Bulman Page, P. C., (2012) Enantioselective total synthesis of (+)-scuteflorin A using organocatalytic asymmetric epoxidation. *Journal of Organic Chemistry* 77, 772-774.

2. Nicolaou, K.C.; Hummel, C. W.; Nakada, M.; Shibayama, K.; Pitsinos, E. N.; Saimoto, H. Mizuno, Y.; Baldenius, K. U.; Smith, A. L., (1993) Total synthesis of calicheamicin .gamma.1I. 3. The final stages. *Journal of the American Chemical Society* 115(17), 7625-7635.