# New class of betulinic acid-based nanoassemblies of cabazitaxel, podophyllotoxin and thiocolchicine

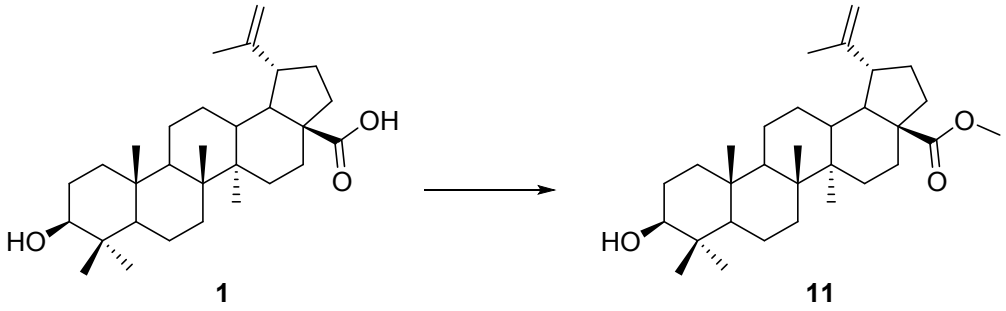
Eleonora Colombo,[a] Laura Polito,[b] Michele Biocotino,[a] Paola Marzullo,[a] Mariafrancesca Hyeraci,[c] Lisa Dalla Via,[c] Daniele Passarella*[,a]

## Chemistry

## General

All reactions were carried out in oven-dried glassware and dry solvents under nitrogen atmosphere.
Unless otherwise stated, all solvents were purchased from Sigma Aldrich and used without further purification.
Substrates and reagents were purchased from Sigma Aldrich and used as received.
Thin layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates.
Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with 1% permanganate solution.
Flash chromatography was performed using silica gel (240-400 mesh, Merck).
$^{1}$H-NMR spectra were recorded on Bruker DRX-400 And Bruker DRX-300 instruments and are reported relative to residual $CDCl_3$.
$^{13}$C-NMR spectra were recorded on the same instruments (101 and 75 MHz) and are reported relative to $CDCl_3$.
Chemical Shifts (δ) for proton and carbon resonances are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard.
MS spectra were recorded using Electrospray ionization (ESI) technique on a Waters Micromass Q-Tof micro mass spectrometer and HR-ESI mass spectra were recorded on FT-ICR $APEX_{II}$ (Bruker Daltonics), EI mass spectra were recorded at an ionizing voltage of 6 kEv on a VG 70-70 EQ.
Specific rotations were measured with a P-1030-Jasco polarimeter with 10 cm optical path cells and 1 ml capacity (Na lamp, $\lambda$ = 589 nm). Microwave assisted reactions were performed with Emrys Creator single-mode (power range 0-400 W from magnetron at 2.45 GHz).
IR spectra were recorded on a Jasco FT-IR 4100 Spectrometer using $CH_2Cl_2$ in NaCl rectangular windows.

## Synthesis of 11




***Reagents and conditions**: trimethylsilyl diazomethane, dry MeOH/PhCH$_3$, rt, 30h.*

Trimethylsilyl diazomethane (2M in n-hexane, 0.66 mL, 1.312 mmol) was added to a solution of betulinic acid (500 mg, 1.093 mmol) in dry MeOH (10 mL) and dry toluene (15 mL). The reaction was stirred overnight at rt, and reaction monitoring (TLC, eluant 7:3 n-hexane/AcOEt with 1% HCOOH) confirmed the disappearance of starting material. The reaction mixture was diluted with diethyl ether (13 mL) and 10% AcOH (10 mL). The aqueous layer was extracted with diethyl ether (3 x 10 mL), and the collected organic phases were washed with sat. $Na_2CO_3$ (10 mL), dried with $Na_2SO_4$ and evaporated under reduced pressure to obtain pure **11** as a white solid (486.1 mg, 1.032 mmol, 95% yield).

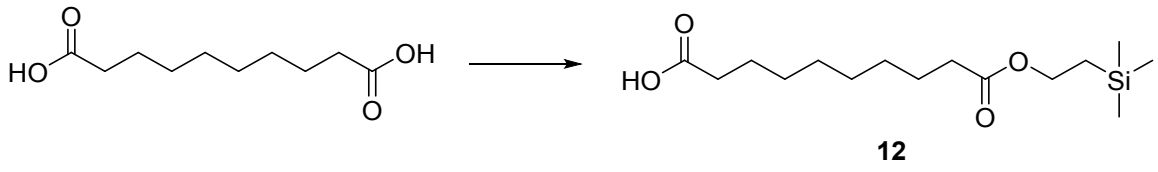
**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 4.63 (bs, 1H), 4.49 (bs, 1H), 3.56 (s, 3H), 3.07 (dd, *J* = 11.2, 5.1 Hz, 1H), 2.89 (td, *J* = 10.9, 4.4 Hz, 1H), 2.20 – 2.02 (m, 2H), 1.77 (dt, *J* = 10.9, 5.9 Hz, 2H), 1.58 (s, 3H), 0.86 (s, 6H), 0.81 (s, 3H), 0.71 (s, 3H), 0.65 (s, 3H).

**$^{13}$C-NMR:** ($CDCl_3$, 75 MHz): δ(ppm) = 176.62, 150.54, 109.49, 78.89, 56.51, 55.32, 54.21, 50.52, 49.43, 46.87, 42.31, 40.60, 38.81, 38.70, 38.23, 37.12, 36.91, 34.28, 32.11, 30.60, 29.61, 27.92, 27.38, 25.48, 20.79, 19.26, 18.31, 16.12, 15.91, 15.33, 14.71.

**$[\alpha]_D^{20}$:** +5.1 (*c*: 1.00, $CHCl_3$).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{31}H_{50}O_3Na$: 493.3658, found 493.3661.

## Synthesis of 12




***Reagents and conditions**: Trimethylsilylethanol, EDC.HCl, DMAP, dry $CH_2Cl_2$, dry Py, rt, 48h.*

Trimethylsilylethanol (313 mL, 2.181 mmol), EDC.HCl (559 mg, 2.909 mmol) and DMAP (89 mg, 0.727 mmol) were added under stirring at rt to a solution of sebacic acid (1 g, 0.4942 mmol) in dry $CH_2Cl_2$ (25 mL)

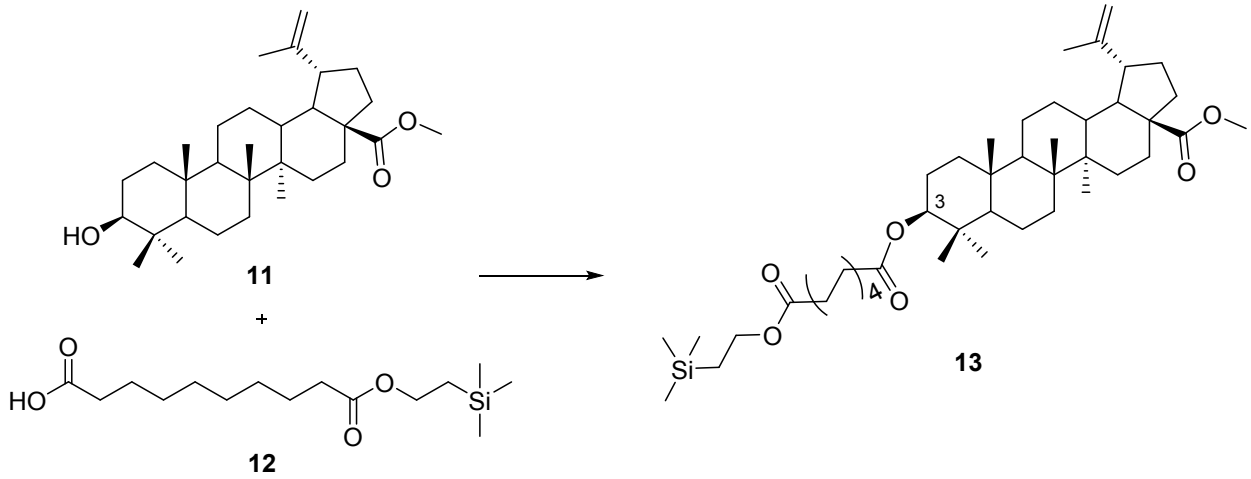
and pyridine (2.5 mL). The reaction mixture was stirred at rt overnight. The reaction mixture was then washed with 10% phosphoric acid (2 x 15 mL) and brine (20 mL). The organic layer was dried with $Na_2SO_4$and evaporated under reduced pressure, and the crude oil was purified by flash chromatography (silicagel, eluant: 8:2 n-hexane/AcOEt with 1% HCOOH) to obtain pure **12** (408 mg, 1,342 mmol, 27% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 4.21-4.12 (m, 2H), 2.38 (t, *J* = 4.5 Hz, 2H), 2.32 (t, *J* = 4.3 Hz, 2H), 1.73-1.59 (m, 4H), 1.41-1.28 (m, 8H), 1.01-0.97 (m, 2H), 0.04 (s, 9H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 177.93, 173.60, 62.58, 34.32, 34.22, 29.46, 29.48, 29.30, 29.10, 25.12, 25.07, 17.05, -1.53 (3C).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{15}H_{30}O_4SiNa$: 325.1811, found 325.1815.

## Synthesis of 13




***Reagents and conditions**: DCC, DMAP, dry $CH_2Cl_2$, rt, 30h.*

Dicyclohexylcarbodiimide (DCC, 201 mg, 0.973 mmol) and dimethylaminopyridine (DMAP, 30 mg, 0.243 mmol) were added under stirring to a solution of compound **11** (229 mg, 0.487 mmol) and compound **12** (221 mg, 0.731 mmol) in dry $CH_2Cl_2$ (5 mL) at 0°C. The reaction is left stirring at rt overnight. Reaction monitoring (TLC, eluant: 9:1 n-hexane/AcOEt) confirmed the disappearance of starting materials. The mixture was diluted with $CH_2Cl_2$(10 mL) and was filtered on a plug of celite. The solvent was removed under reduced pressure, and the resulting crude oil was purified by flash chromatography (silicagel, eluent: 96:4 n-hexane/AcOEt) to obtain pure **13 (**339 mg, 0.449 mmol, 92% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 4.74 (bs, 1H), 4.61 (bs, 1H), 4.48 (dd, *J* = 10.1, 6.2 Hz, 1H), 4.21-4.12 (m, 2H), 3.67 (s, 3H), 3.06-2.95 (m, 1H), 2.33-2.13 (m, 6H), 1.97-1.82 (m, 2H), 1.69 (s, 3H), 1.47-1.34 (m, 8H), 0.97 (s, 3H), 0.92 (s, 3H), 0.85 (s, 3H), 0.84 (s, 6H), 0.05 (s, 9H).

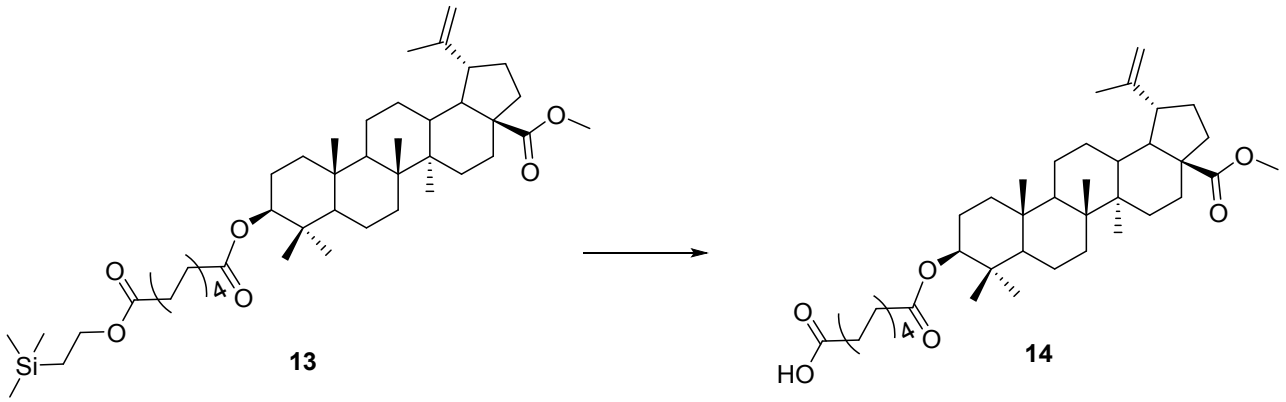
**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 176.67, 174.00, 173.64, 150.57, 109.63, 80.61, 62.37, 56.57, 55.45 (2C), 51.25, 50.46, 49.48, 47.01, 42.40, 40.70, 38.40, 38.27, 37.85, 37.12, 36.98, 34.82, 34.52, 34.27, 32.18,

30.61, 29.68, 29.10 (3C), 27.97, 25.49, 25.12, 24.95, 23.75, 20.91, 19.36, 18.19, 17.33, 16.57, 16.18, 15.96, 14.69, -1.47 (3C).

$[\alpha]_D^{20}$: +10.2 (*c*: 1.00, $CHCl_3$).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{46}H_{78}O_6SiNa$: 777.5465, found 777.5469.

## Synthesis of 14




***Reagents and conditions**: TBAF, dry THF, rt, 20h.*

Tetrabutylammonium fluoride (TBAF, 0.61 mL, 2.11 mmol) was added under stirring to a solution of compound **13** (318 mg, 0.421 mmol) in dry THF (15 mL), and the reaction mixture was stirred at rt overnight. The reaction was quenched by addition of sat. $NH_4Cl$ (10 mL). The aqueous phase was extracted with AcOEt (2 x 10 mL), the collected organic phases were dried with $Na_2SO_4$ and evaporated under reduced pressure to obtain pure **14** (258 mg, 0.393 mmol, 93% yield).

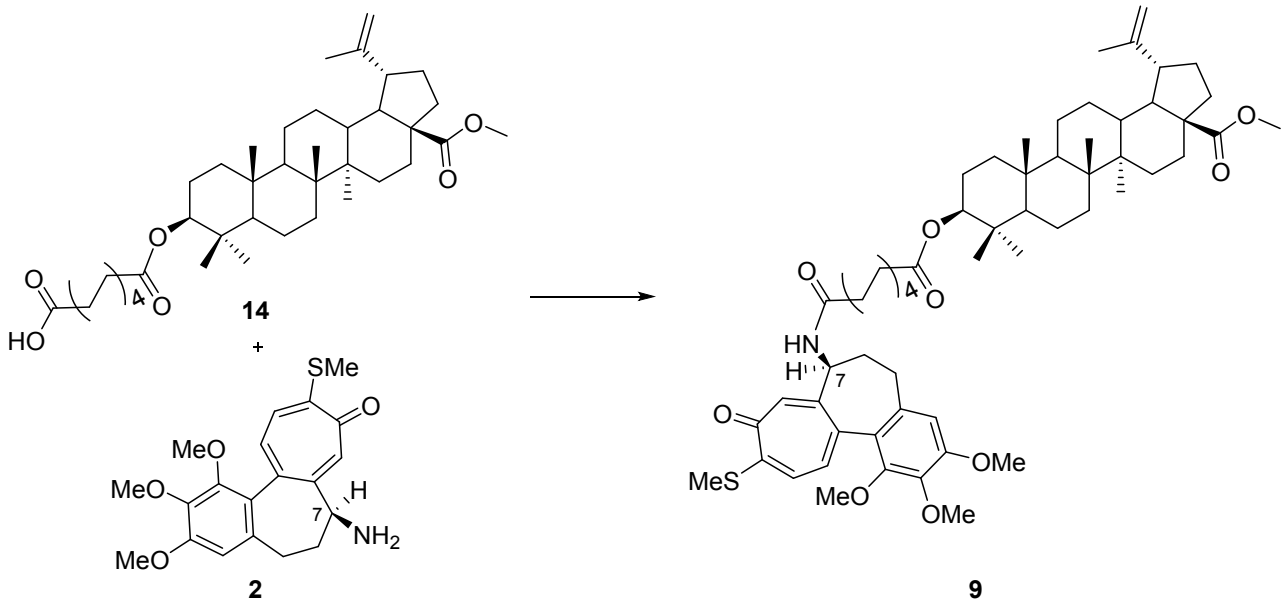
**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 4.72 (bs, 1H), 4.58 (bs, 1H), 4.44 (dd, *J* = 10.1, 6.2 Hz, 1H), 3.65 (s, 3H), 3.03-2.94 (m, 1H), 2.23-2.16 (m, 6H), 1.92-1.82 (m, 2H), 1.67 (s, 3H), 1.47-1.34 (m, 8H), 0.95 (s, 3H), 0.90 (s, 3H), 0.84 (s, 3H), 0.82 (s, 6H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 180.32, 177.30, 174.33, 151.11, 110.29, 81.31, 57.20, 56.08 (2C), 51.89, 51.10, 50.12, 47.63, 43.04, 41.34, 39.04, 38.90, 38.47, 37.76, 37.60, 35.43, 34.92, 34.70, 32.81, 31.25, 30.32, 29.68 (3C), 28.61, 26.13, 25.73, 25.29, 24.38, 21.56, 20.00, 18.84, 17.21, 16.81, 16.59, 15.33.

$[\alpha]_D^{20}$: +12.9 (*c*: 1.00, $CHCl_3$).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{41}H_{66}O_6Na$: 677.4757, found 677.4761.

## Synthesis of 9




***Reagents and conditions:*** *DCC, DMAP, dry $CH_2Cl_2$, rt, 24h.*

Dicyclohexylcarbodiimide (DCC, 63 mg, 0.304 mmol) and dimethylaminopyridine (DMAP, 9 mg, 0.076 mmol) were added under stirring to a solution of compound **14** (99 mg, 0.152 mmol) in dry $CH_2Cl_2$ (5 mL) at 0°C. Subsequently N-desacetylthiocolchicine **2** was added (56 mg, 0.152mmol) and the reaction mixture was stirred at rt overnight. The mixture was diluted with $CH_2Cl_2$ (10 mL) and was filtered on a plug of celite. The solvent was removed under reduced pressure, and the resulting crude was purified by flash chromatography (silicagel, eluent: 99:1 $CH_2Cl_2$/MeOH) to obtain pure **9 (**118 mg, 0.116 mmol, 77% yield).

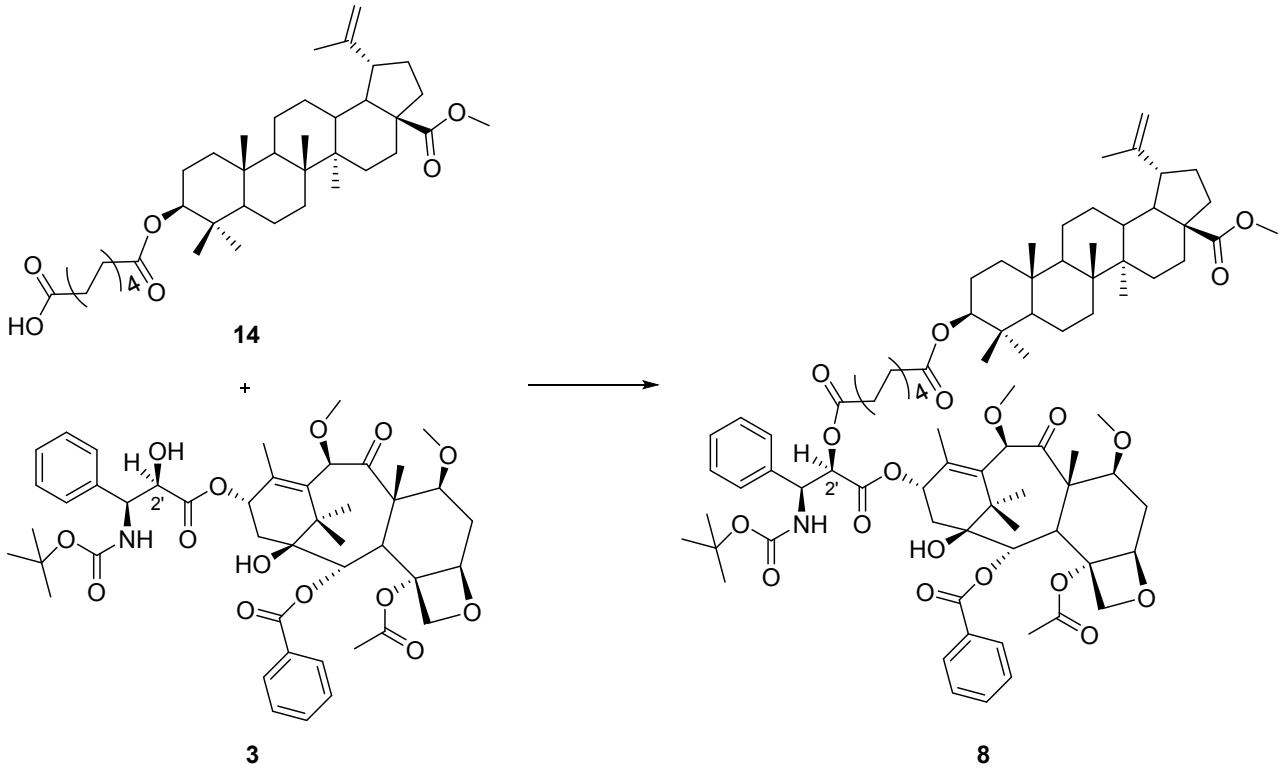
**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 7.31 (d, *J* = 5.6 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.06 (d, *J* = 10.4 Hz, 1H), 6.81 (d, *J* = 7.1 Hz, 1H), 6.52 (s, 1H), 4.73 (s, 1H), 4.71 – 4.63 (m, 1H), 4.59 (s, 1H), 4.45 (m, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.66 (s, 6H), 2.99 (m, 1H), 2.57 – 2.48 (m, 1H), 2.46 – 2.33 (m, 4H), 2.30 – 2.13 (m, 8H), 1.95 – 1.78 (m, 3H), 1.74 – 1.64 (m, 5H), 1.62 – 1.45 (m, 7H), 1.44 – 1.30 (m, 8H), 1.30 – 1.17 (m, 17H), 0.95 (s, 3H), 0.90 (s, 3H), 0.87 – 0.72 (m, 6H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) =182.46, 176.78, 173.78, 172.91, 158.33, 153.73, 151.41, 150.66, 141.86, 138.44, 134.74, 134.46, 128.67, 126.60, 125.90, 109.74, 107.54, 80.71, 61.80, 61.50, 56.68, 56.24 (2C), 55.56, 51.95, 51.35, 50.57, 49.59, 47.12, 42.51, 40.81, 38.51, 38.38, 37.95, 37.23, 37.07, 36.52, 34.93, 34.38, 32.28, 31.62, 30.72, 30.12, 29.79 (3C), 29.35, 29.22 (2C), 28.09, 25.59, 25.23, 23.86, 21.01, 19.46, 18.30, 16.68, 16.27, 16.07, 15.26, 14.80.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: -84.4 (*c*: 1.00, $CHCl_3$).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{61}H_{87}NO_9SNa$: 1032.5999, found 1032.6002.

## Synthesis of 8




***Reagents and conditions**: DCC, DMAP, dry $CH_2Cl_2$, 25 °C, 25h.*

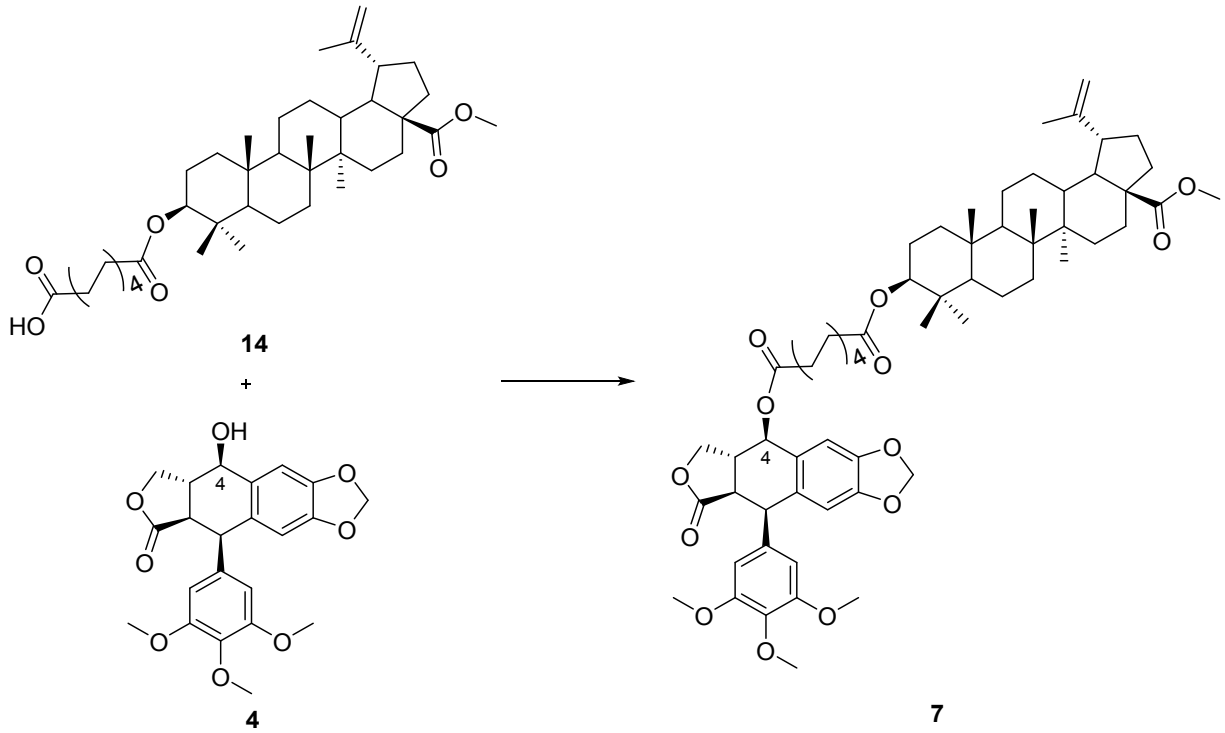
Dicyclohexylcarbodiimide (DCC, 69 mg, 0.332 mmol) and dimethylaminopyridine (DMAP, 10 mg, 0.083 mmol) were added under stirring to a solution of compound **14** (109 mg, 0.166 mmol) in dry $CH_2Cl_2$ (5.5 mL) at 0°C. Subsequently cabazitaxel **3** (32 mg, 0.0383 mmol) was added. The reaction mixture was stirred at rt overnight. The mixture was diluted with $CH_2Cl_2$ (10 mL) and was filtered on a plug of celite. The solvent was removed under reduced pressure, and the resulting crude was purified by flash chromatography (silicagel, eluent: 7:3 n-hexane/AcOEt) to obtain pure **8 (**54 mg, 0.0363 mmol, 95% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 7.98 (d, *J* = 7.0 Hz, 1H), 7.84 (s, 1H), 7.74 (t, *J* = 7.3 Hz, 1H), 7.66 (t, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.37 (d, *J* = 7.6 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 5.78 (d, *J* = 19.4 Hz, 1H), 5.57 (d, *J* = 7.9 Hz, 1H), 5.37 (d, *J* = 7.1 Hz, 1H), 5.06 (d, *J* = 7.0 Hz, 2H), 4.95 (d, *J* = 8.4 Hz, 1H), 4.73 – 4.67 (m, 2H), 4.58 (t, *J* = 1.9 Hz, 1H), 4.38 (dd, *J* = 11.4, 4.9 Hz, 1H), 4.02 (s, 2H), 3.75 (dd, *J* = 10.6, 6.7 Hz, 1H), 3.60 (s, 3H), 3.39 – 3.26 (m, 6H), 3.22 (s, 3H), 2.92 (m, *J* = 10.7, 5.1 Hz, 1H), 2.72 – 2.60 (m, 1H), 2.38 (t, *J* = 7.3 Hz, 2H), 2.28 (m, 1H), 2.24 (s, 3H), 2.15 (td, *J* = 12.4, 3.5 Hz, 2H), 1.84 – 1.67 (m, 9H), 1.68 – 1.57 (m, 6H), 1.59 – 1.44 (m, 10H), 1.38 (s, 8H), 1.38 – 1.28 (m, 3H), 1.32 – 1.24 (m, 3H), 1.24 (s, 9H), 1.22 – 1.04 (m, 3H), 1.08 – 1.01 (m, 1H), 1.05 – 0.93 (m, 9H), 0.85 (s, 3H), 0.83 – 0.77 (m, 9H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 204.60, 175.64, 172.49, 172.24, 169.64, 169.13, 165.20, 156.58, 155.13, 150.05, 138.44, 137.43, 134.92, 133.43, 129.92, 129.54, 128.63, 128.52, 128.03, 127.39, 109.75, 83.23, 82.00, 80.28, 80.11, 79.70, 78.39, 76.70, 75.19, 74.93, 74.34, 70.98, 56.59, 56.48, 55.94, 55.86, 55.11, 54.60, 51.18, 49.61, 48.72, 47.48, 46.63, 46.36, 42.88, 41.97, 37.71, 37.65, 37.36, 36.60, 36.13, 34.42, 33.92,

33.65, 33.32 (2C), 33.13, 31.65, 31.42, 29.97, 29.14, 28.41 (2C), 28.33, 28.09, 27.62, 26.58, 25.30 (2C), 24.97, 24.56, 24.43 (2C), 24.25, 23.36, 22.49, 21.09, 20.40, 18.88, 17.70, 16.41, 15.80, 15.59, 14.34, 14.00, 10.09.
$[\alpha]_D^{20}$: -29.1 (*c*: 1.00, $CHCl_3$).
**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{86}H_{121}NO_{19}Na$: 1494.8431, found 1494.8432.

## Synthesis of 7




***Reagents and conditions**: DCC, DMAP, dry $CH_2Cl_2$, rt, 24h.*

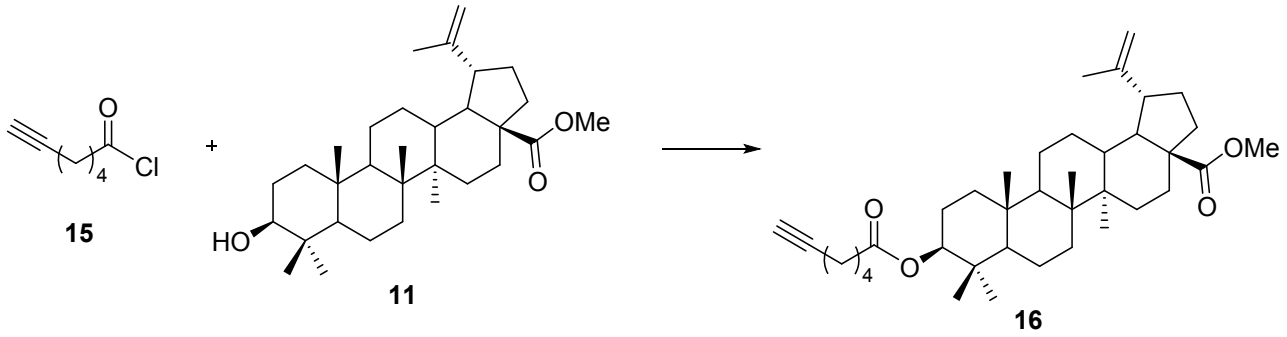
Dicyclohexylcarbodiimide (DCC, 63 mg, 0.304 mmol) and dimethylaminopyridine (DMAP, 9 mg, 0.076 mmol) were added under stirring to a solution of compound **14** (100 mg, 0.152 mmol) in dry $CH_2Cl_2$ (4 mL) at 0°C. Subsequently podophyllotoxine **4** (63 mg, 0.152 mmol) was added. The reaction mixture was stirred at rt overnight. The mixture was diluted with $CH_2Cl_2$ (10 mL) and was filtered on a plug of celite. The solvent was removed under reduced pressure, and the resulting crude was purified by flash chromatography (silicagel, eluent: 8:2 n-hexane/AcOEt) to obtain pure **7** (106 mg, 0.101 mmol, 66% yield).
**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 6.76 (s, 1H), 6.55 (s, 1H), 6.40 (s, 2H), 6.02 – 5.96 (m, 2H), 5.89 (d, *J* = 9.1 Hz, 1H), 4.74 (s, 1H), 4.61 (s, 2H), 4.52 – 4.44 (m, 1H), 4.40 – 4.34 (m, 1H), 4.21 (t, *J* = 9.8 Hz, 1H), 3.82 (s, 3H), 3.77 (s, 6H), 3.68 (s, 3H), 3.06 – 2.93 (m, 1H), 2.92 (d, *J* = 4.4 Hz, 1H), 2.88 – 2.75 (m, 1H), 2.49 – 2.36 (m, 2H), 2.33 – 2.16 (m, 4H), 1.95 – 1.82 (m, 2H), 1.75 – 1.54 (m, 12H), 1.53 – 1.19 (m, 24H), 1.18 – 1.01 (m, 2H), 0.97 (s, 3H), 0.92 (s, 3H), 0.85 (s, 6H).
**$^{13}$C-NMR:** ($CDCl_3$, 100 MHz): δ(ppm) = 177.33, 174.84, 174.31, 153.33, 151.21, 148.79, 148.27, 137.91, 135.50, 133.01, 129.13, 110.39, 110.28, 108.86 (2C), 107.65, 102.25, 81.32, 74.08, 72.04, 61.40, 57.22, 56.82

(2C), 56.12, 51.89, 51.13, 50.15, 47.67, 46.26, 44.43, 43.06, 41.37, 39.44, 39.06, 38.93, 38.50, 37.79, 37.62, 35.41, 35.02, 34.93, 32.83, 32.10, 31.27, 30.86, 30.34 (2C), 29.74 (3C), 28.63, 26.15, 25.73, 25.61, 24.42, 21.58, 20.01, 18.86, 17.23, 16.82, 16.61, 15.34.

$[\alpha]_D^{20}$: -41.3 (*c*: 1.00, $CHCl_3$).

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{63}H_{86}O_{13}Na$: 1073.5966, found 1073.5969.

## Synthesis of 16




***Reagents and conditions**: DIPEA, PPy, dry $CH_2Cl_2$, 0°C to rt, 30h.*

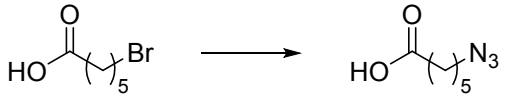
To a solution of **11** (88.4 mg, 0.19 mmol) in dry $CH_2Cl_2$ (0.95 mL), DIPEA (36 µL) and 4-(1-pyrrolidinyl)pyridine (28 mg, 0.19 mmol) were added at 0°C. Then **15** (27 µL, 0.19 mmol) was slowly added and the mixture was stirred overnight. Another quantity of DIPEA (36 µL, 0.21 mmol) and **15** (27 µL, 0.19 mmol) were added at 0°C, and the reaction was stirred for another 5h. The reaction was stopped adding 1 mL of water, and the aqueous phase was extracted with $CH_2Cl_2$ (3 x 1 mL). The combined organic phases were washed with $NaHCO_3$ (2 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure. The obtained crude was purified by flash chromatography (silicagel, eluant 8:2 n-hexane/AcOEt) to obtain **16** (85.7 mg, 0.152 mmol, 80% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 4.74 (d, *J* = 2.4 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.53 – 4.46 (m, 1H), 3.67 (s, 3H), 3.00 (td, *J* = 10.8, 4.3 Hz, 1H), 2.33 (t, *J* = 7.4 Hz, 2H), 2.22 (td, *J* = 7.0, 2.8 Hz, 4H), 1.98 – 1.82 (m, 3H), 1.82 – 1.71 (m, 4H), 1.69 (s, 3H), 1.67 – 1.53 (m, 6H), 1.50 (dd, *J* = 6.0, 2.8 Hz, 1H), 1.46 – 1.32 (m, 9H), 1.32 – 1.21 (m, 4H), 1.15 (dd, *J* = 9.7, 2.9 Hz, 1H), 0.97 (s, 3H), 0.92 (s, 3H), 0.89 – 0.82 (m, 9H), 0.79 (d, *J* = 9.9 Hz, 1H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 176.62, 173.14, 150.51, 109.63, 80.80, 68.57, 56.55, 55.44, 51.23, 50.46, 49.47, 46.99, 42.39, 40.70, 38.38, 38.25, 37.83, 36.95, 34.26, 34.22, 32.16, 30.60, 29.67, 27.99, 27.87, 25.48, 24.17, 23.74, 20.90, 19.35, 18.18, 18.12, 16.56, 16.16, 15.95, 14.68.

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{38}H_{58}O_4Na$: 601.4233, found 601.4237.

## Synthesis of 17




**17**

***Reagents and conditions**: NaN3, dry DMSO, rt, 12h.*

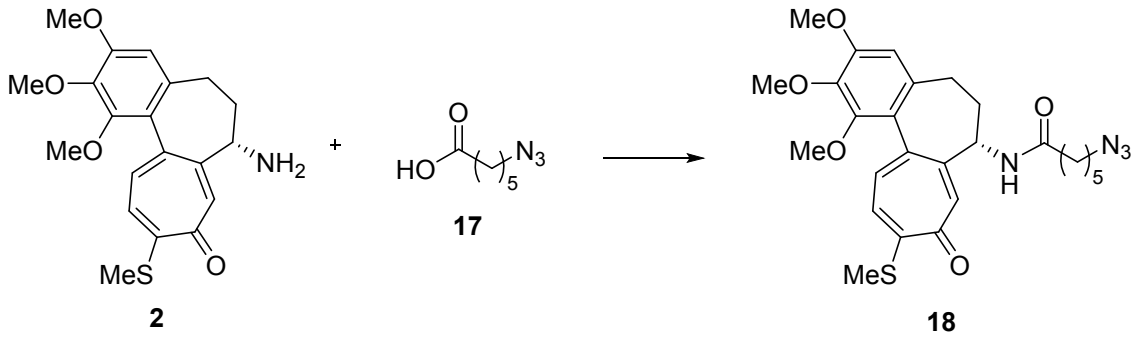
To a solution of 6-bromo-hexanoic acid (150 mg, 0.769 mmol) in DMSO (3 mL), $NaN_3$ (55 mg, 0.846 mmol) was added. The reaction mixture was stirred at room temperature for 12h. Water (6 mL) was added and aqueous phase was extracted with AcOEt (4 x 6 mL). The combined organic phases were washed twice with water, then with brine. The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The colourless crude product was used in the next step without further purification (118.8 mg, 0.723 mmol, 94% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 3.30 (t, *J* = 6.9 Hz, 2H), 2.39 (t, *J* = 7.4 Hz, 2H), 1.66 (dp, *J* = 18.1, 7.3 Hz, 4H), 1.45 (m, 2H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 180.19, 51.87, 34.49, 29.20, 26.82, 24.83.

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_6H_{11}N_3O_2Na$: 180.0749, found 180.0752.

## Synthesis of 18




***Reagents and conditions**: DCC, DMAP, dry $CH_2Cl_2$, 0°C to rt, 24h.*

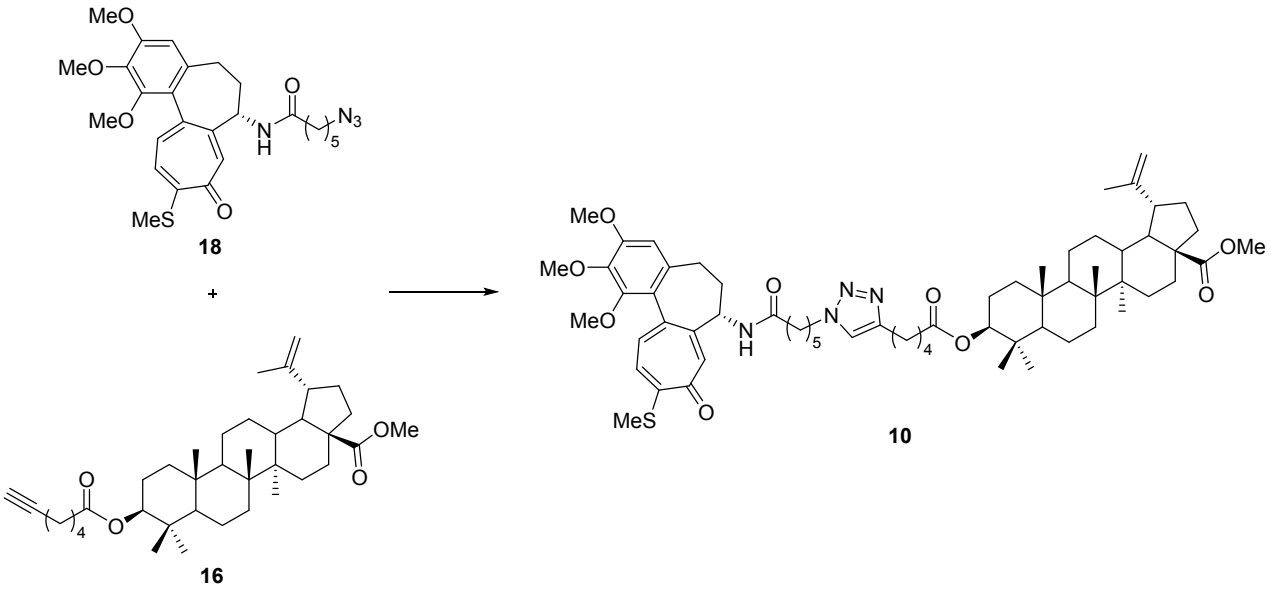
To a stirred solution of **2** (188.0 mg, 0.503 mmol) in dry $CH_2Cl_2$ (3.4 mL) at 0°C were added **17** (78.0 mg, 0.503 mmol), DCC (134.9 mg, 0.654 mmol) and DMAP (30.6 mg, 0.252 mmol). After 30 minutes the reaction mixture was brought at room temperature and stirred overnight. Afterwards, the solution was filtered and washed with an aqueous saturated solution of $NaHCO_3$ (1 x 3 mL). The aqueous phase was extracted with $CH_2Cl_2$ (2 x 3 mL) and the organic layer was dried over $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silicagel, eluant from $CH_2Cl_2$ to 95:5 $CH_2Cl_2$/MeOH) to gain a yellow solid (396.3 mg, 0.503 mmol, quant. yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 7.52 (d, *J* = 7.4 Hz, 1H), 7.43 (s, 1H), 7.31 (t, *J* = 12.1 Hz, 1H), 7.10 (d, *J* = 10.4 Hz, 1H), 6.55 (s, 1H), 4.71 (dt, *J* = 12.5, 6.7 Hz, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 3.68 (s, 3H), 3.24 (t, *J* = 7.0 Hz, 2H), 2.54 (dd, *J* = 13.5, 6.2 Hz, 1H), 2.45 (s, 3H), 2.38 (dd, *J* = 13.2, 6.5 Hz, 1H), 2.27 (m, 3H), 1.99 – 1.80 (m, 1H), 1.80 – 1.46 (m, 4H), 1.46 – 1.19 (m, 2H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 183.06, 173.23, 158.85, 154.30, 152.41, 151.89, 142.34, 139.18, 135.40, 135.07, 129.26, 127.32, 126.38, 108.07, 62.35, 62.06, 56.78, 52.59, 51.91, 49.71, 37.46, 36.69, 30.69, 29.20, 27.02, 25.72, 15.81.

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{26}H_{32}N_4O_5SNa$: 535.1991, found 535.2000.

## Synthesis of 10




***Reagents and conditions**: $CuSO_4 5H_2O$, sodium ascorbate, DABCO, AcOH, $H_2O$/t-BuOH, rt, 3h.*

To a solution of $CuSO_4 \cdot 5H_2O$ (1.1 mg, 0.004 mmol) in $H_2O$/t-BuOH 1.2:1 (550µL), sodium ascorbate (3.6 mg, 0.018 mmol), DABCO (1.36 mg, 0.012 mmol), a drop of glacial acetic acid, **16** (85.7 mg, 0.151 mmol) and **18** (77.2 mg, 0.151 mmol) were added. The reaction mixture was stirred at room temperature for 3h. Subsequently, the reaction was diluted with AcOEt (1 mL), an aqueous saturated solution of $NH_4Cl$ (1 mL) was added and the resulting mixture was vigorously stirred for 30 min. The aqueous phase was extracted with AcOEt (3 x 2 mL). The combined organic phase was dried over $Na_2SO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (silicagel, eluant: from $CH_2Cl_2$ to 97:3 $CH_2Cl_2$/MeOH) providing **10** as yellow solid (82.7 mg, 0.077 mmol, 51% yield).

**$^1$H-NMR:** ($CDCl_3$, 400 MHz): δ(ppm) = 7.48 (s, 1H), 7.33 – 7.27 (m, 2H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.08 (d, *J* = 10.5 Hz, 1H), 6.55 (s, 1H), 4.74 (d, *J* = 2.4 Hz, 1H), 4.66 (dd, *J* = 12.1, 6.4 Hz, 1H), 4.61 (d, *J* = 3.3, 1.9 Hz, 1H), 4.46 (dd, *J* = 9.3, 7.0 Hz, 1H), 4.40 – 4.19 (m, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.68 (s, 6H), 3.00 (td, *J* = 10.9, 4.4 Hz, 1H), 2.74 (td, *J* = 7.1, 2.7 Hz, 2H), 2.54 (dd, *J* = 13.5, 6.4 Hz, 1H), 2.45 (m, 4H), 2.34 (dd, *J* =

7.9, 5.9 Hz, 2H), 2.23 (m, 5H), 1.89 (m, 4H), 1.71– 1.53 (m, 11H), 1.52 – 1.46 (m, 1H), 1.48 – 1.20 (m, 13H), 1.19 – 1.13 (m, 1H), 0.97 (s, 4H), 0.93 (s, 3H), 0.85 (s, 3H), 0.83 (s, 6H), 0.81 – 0.74 (m, 1H).

**$^{13}$C-NMR:** ($CDCl_3$, 101 MHz): δ(ppm) = 182.32, 176.66, 173.58, 172.44, 158.22, 153.64, 151.25, 150.53, 147.42, 141.75, 138.30, 134.67, 134.31, 128.54, 126.49, 125.78, 121.39, 109.63, 107.44, 80.90, 61.67, 61.38, 56.56, 56.14, 55.50, 52.11, 51.25, 50.48, 49.94, 49.49, 47.00, 42.40, 40.71, 38.40, 38.26, 37.84, 37.13, 36.97, 36.67, 35.91, 34.49, 34.27, 32.18, 30.61, 30.02, 29.68, 28.81, 28.02, 26.10, 25.48, 25.28, 24.95, 24.55, 23.77, 20.91, 19.36, 18.20, 16.56, 16.16, 15.14, 14.70.

**HRMS (ESI):** (m/z) $[M+Na]^+$ calcd. for $C_{64}H_{90}N_4O_9SNa$: 1113.6326, found 1113.6335.

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$: -70.7 (*c*: 0.80, $CHCl_3$).

**Nanoparticles assembly**

In accordance with standard solvent evaporation protocols the conjugate (4.0 mg) was first dissolved in THF (1 mL) in a vial while stirring at rt. The resulting solution was added dropwise to a round bottom flask containing MilliQ grade distilled water (2 mL) under magnetic stirring (500 rpm). The resulting suspension was stirred for 5 min, then THF was thoroughly evaporated under reduced pressure, obtaining pure NPs as an opalescent suspension (2 mL, 2 mg/mL).

**Nanoparticles characterization**

NPs were characterized by dynamic light scattering (DLS), using a 90 Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY, USA) operating at 15 mW of a solid-state laser (λ = 661 nm), using a 90-degree scattering angle. The ζ-potential was determined at 25 °C using a 90 Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY, USA) equipped with an AQ-809 electrode, operating at an applied voltage of 120 V. Ten independent measurements of 60 s duration were performed for each sample. Hydrodynamic diameters were calculated using Mie theory, considering the absolute viscosity and refractive index values of the medium to be 0.890 cP and 1.33, respectively. The same aqueous samples at a concentration of 0.2 mg/mL were used for ζ-potential measurement, without any change for the ionic strength (no addition of KCl). The ζ-potential was calculated from the electrophoretic mobility of nanoparticles, by using the Smoluchowski theory.

## Biology

## Inhibition growth assay

A2780 (human ovarian cancer) cells were grown in RPMI 1640 supplemented with 1.5 g/L $NaHCO_3$, 10% heat-inactivated foetal bovine serum (Biowest), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Sigma Chemical Co.). Cells were seeded into 24-well cell culture plates ($2.5 \times 10^4$

cells/well) and incubated for 24 h. Test compounds were added to the complete medium at different concentrations and the cells were incubated for a further 72 h. The building blocks were dissolved at 5 mM in ethanol and the final volume of solvent in each sample did not exceed 0.4%. Cell viability was determined by the trypan blue exclusion assay and $GI_{50}$ values, i.e. the concentration (μM) of building blocks or nanoparticles that induces 50% reduction in cell number with respect to control cultures, were calculated.

## Analysis of DNA content

A2780 cells were plated into 6-well cell culture plates (about $5 \times 10^5$ cells /well) and incubated for 24 h of in a drug-free medium. The building block or nanoparticle was then added to the complete medium at the indicated concentrations and the cells were incubated for a further 24 h. After treatment, the cells were harvested, samples of $10^6$ cells per sample were fixed in 70% iced-cold ethanol at -20 °C for 20 min and then washed with phosphate buffered saline (PBS). The obtained cell pellet was gently resuspended in PBS in the presence of 0.1 mg/mL RNAse and 38 μg/mL propidium iodide. The cell suspension was incubated for 20 min in the dark at room temperature until measured. The analysis of DNA fluorescence was measured by flow cytometry on a FACSCanto II flow cytometer (Becton–Dickinson, Mountain View, CA).