Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

TABLE OF CONTENTS *(to be defined)*

1. Materials and Methods

1. I. Products

All organic solvents were purchased from commercial suppliers and used without further purification or drying. DMF extra dry (with molecular sieves, water < 50 ppm) was acquired from ACROS Organics. DMF an NMP (peptide synthesis grade) were purchased from Biosolve. HPLC grade quality Hexane and Chloroform were purchased from Fisher Scientific. Ethyl Acetate, Acetonitrile, Methanol, Diethyl Ether DIPEA, supplied as extra dry, redistilled, 99.5 % pure and Triisopropylsilane were purchased from Sigma Aldrich. Deuterated solvents $CDCl_3$ (99.8% atom D) and CD_3OD (99.8% atom D) were obtained from Sigma-Aldrich. Water with the Milli-Q grade standard was obtained in-house either from a Millipore ROs 5 purification system or a Sartorius Arium 611 DI. Rink-Amide ChemMatrix (100-200 µm, manufacturer's loading: 0.52-0.54 mmol/g) was obtained from Biotage. All reagents were acquired from commercial sources and used without prior purification.

Fmoc-Propargylglycine-OH, Fmoc-Azidohomoalanine-OH, PyBOP and HBTU coupling reagents were obtained from either Merck Novabiochem or IRIS Biotech GmbH, while HATU (purum ≥ 98.0 %) was acquired from Fluka. TFA was purchased from Iris Biotech GmbH. NHS-Fluorescein was purchased from Thermo Scientific. The Nα-Fmoc protected amino acids were purchased at Merck Novabiochem, IRIS Biotech GmbH and Fluka, or supplied by MultiSynTech GmbH. All chiral α-amino acids used in this paper possessed the L configuration. Throughout this work, residues with standard acid-sensitive side-chain PGs were used: Cys(Trt) [C], Asp(OtBu) [D], Glu(OtBu) [E], Lys(Boc) [K], Asn(Trt) [N], Gln(Trt) [Q], Arg(Pbf) [R], Ser(tBu) [S], Thr(tBu) [T].

Oligonucleotides used were commercially purchased from Eurogentec (HPLC purified using RP-cartridge-Gold, 200 nm scale) and Sigma Aldrich ()

1. II. Peptide Synthesis

Automated peptide syntheses were performed on a fully-automated SYRO Multiple Peptide Synthesizer robot, equipped with a vortexing unit for the 24-reactor block (MultiSynTech GmbH). Reactions were open to the atmosphere and executed at ambient temperature.

1. III. Analysis & Equipment

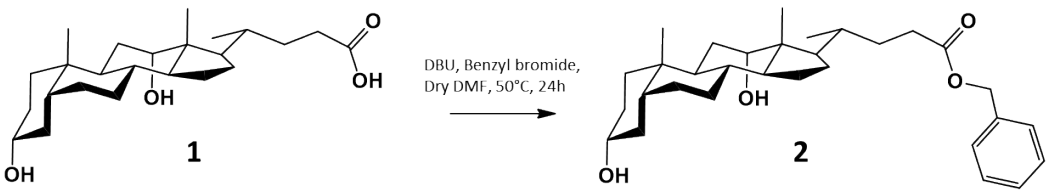
Reversed-Phase HPLC analysis and purification was performed on an Agilent 1100 Series instrument with diode array detector (at 214, 254, 280, 310, 360 nm), equipped with a Phenomenex Luna C18(2) 100 Å column (250 x 4.6 mm, 5 µm, at 35 °C) or a Phenomenex Jupiter C4 300 Å column (250 x 4.6 mm, 5 µm, at 35 °C). Linear gradient elutions were performed by flushing 2 min with A followed by 0 to 100% buffer B in 15 minutes and finally by a 5 min flushing with B using a binary solvent system composed of buffer A: 0.1% TFA in H_2O and B: MeCN) with a flow of 1.0 mL/min at 35°C. ESI-MS spectra were recorded on a quadrupole ion trap LC mass spectrometer (Thermo Finnigan MAT LCQ), equipped with electrospray ionization. MeOH/H_2O (4/1 ± 0.1 % formic acid) was used as carrier solution. All reported data were collected in the positive mode, at 250 °C. MALDI-TOF-MS data were acquired on an Applied

Biosystems Voyager-DE STR Biospectrometry Workstation, equipped with a high performance nitrogen laser (337 nm). All spectra were recorded in the positive and reflector mode, with delayed extraction. LC-TIC-MS data (reversed phase) were recorded on an Agilent 1100 Series instrument with diode array detector (at 214, 254, 280, 310, 360 nm), equipped with a Phenomenex Luna C18 100 Å column (250 mm x 4.6 mm, 5 µm, at 35 °C) or Phenomenex Kinetex C18 100 Å (150 x 4.6 mm, 5 µm, at 35 °C), hyphenated to an Agilent ESI-single quadrupole MS detector type VL. Mass detection operated in the positive mode. Linear gradient elutions were performed by using a binary solvent system composed of buffer A: 0.1% formic acid in H_2O and B: MeCN) with a 1.0 mL/min flow. Pre- and postflushing is included in the LC-MS and RPHPLC results. A solution of 4-5 mg α-cyano-4-hydroxycinnamic acid in 500µl MeCN, 490µl mQ, 10µl 1M ammoniumcitrate, 1µl TFA was used as matrix for MALDI-TOF-MS. Concentrations were determined by ^{1}H-NMR ERETIC (Electronic Reference To access In vivo Concentrations)[1] with a high-resolution 500 MHz NMR-spectrometer.

NMR spectra were recorded at room temperature on a Bruker Avance-300 spectrometer at 300 MHz for ^{1}H NMR and on a Bruker Avance-500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C spectra. Deuterated solvents $CDCl_3$ (99.8% atom D) and CD_3OD (99.8% atom D) were obtained from Sigma-Aldrich. Chemical shifts (δ units) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) and the internal solvent peak was used for calibration. When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constants (J values) are expressed in Hertz (Hz). The Attached Proton Test (APT) technique was used to assign ^{13}C peaks (C, CH, CH_2, CH_3). All ^{1}H spectra of deoxycholic acid derived products contain a region of high signal overlap between 1.00 and 2.00 ppm. Therefore, the detailed analysis of this envelop is impossible and often not mentioned or reported as a series of multiplets. Fortunately, in the preparation of scaffolds all necessary information was shifted downfield from this region.

2. Scaffold Synthesis

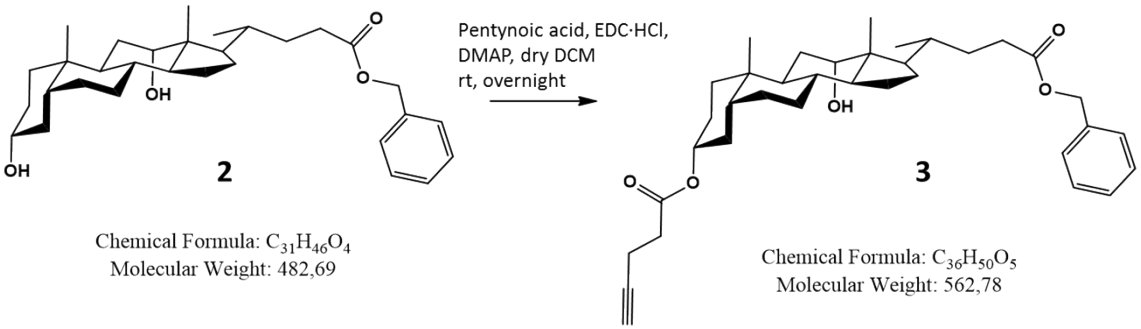
Benzyl 3α, 12α-dihydroxy-5β-cholan-24-oate. To a solution of deoxycholic acid **1** (3 g, 7.642 mmol) in DMF (7 mL), DBU (1.38 mL, 9.25 mmol) and benzyl bromide (1.1 mL, 9.25 mmol) were added and the mixture was stirred at 50 °C for 24 h. DMF was removed under vacuum, 10 % HCl (80 mL) was added to the residue and the product was extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water (100 mL) and aq. $NaHCO_3$ (100 mL) and dried over anhyd. Na_2SO_4. The crude product obtained after removal of the solvent was column chromatographed on silica gel using 5 % EtOAc/$CHCl_3$ (R_f: 0.38) to yield 3.8 g (78 %) of compound **2** as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.36 − 7.38 (m, 5 H), 5.05 (d, J = 1.8 Hz, 2 H), 3.92 (s, 1 H), 3.40 (br, m, 3 H), 2.45 – 2.36 (m, 1 H), 2.30 – 2.20 (m, 1 H), 2.21 (t, J = 12 Hz, 2 H), 0.90 (d, J = 6.28 Hz, 3 H), 0.80 (s, 3 H), 0.63 (s, 3 H). ES-MS m/z (% rel. int.) 541.3 (100) [M + OAc^-].

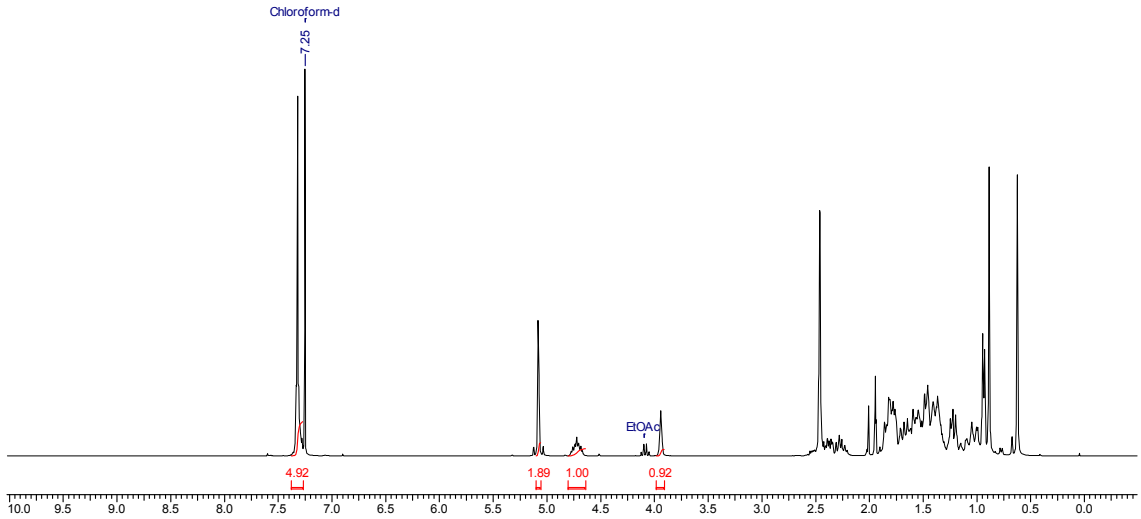


Chemical Formula: $C_{24}H_{40}O_4$
Molecular Weight: 392,57

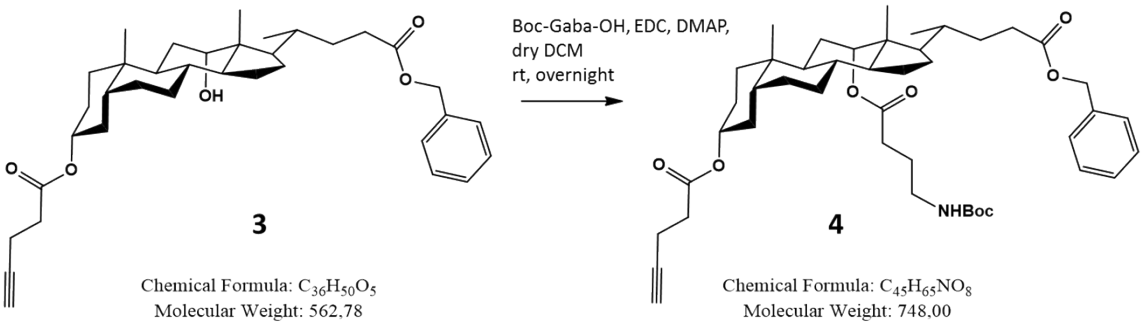
Chemical Formula: $C_{31}H_{46}O_4$
Molecular Weight: 482,69

Benzyl 3α-pentynoate-5β-cholan-24-oate. Compound **2** (500 mg, 1.037 mmol) was dissolved in 2 mL dry DCM. Then a solution of 4-pentynoic acid (122.1 mg, 1.24 mmol) and DMAP (12.7 mg, 0.104 mmol) in dry DCM (2.2 mL) was added and stirred for 10 min in an ice bath. Then, EDC·HCl (298.3 mg, 1.55 mmol) in 1 mL dry DCM was added portion-wise to the reaction mixture and stirred for 30 min at 0°C, then the reaction mixture was stirred at room temperature overnight. The residue was purified by column chromatography (silica gel EtOAc/Hexane 1:3; R_f: 0.33) to give **3** as a yellow oil (60% yield). ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.38 − 7.36 (m, 5 H), 5.05 (d, J = 1.8 Hz, 2 H), 4.75 (br, m, 1 H), 3.9 (m, 1H), 2.5 (s, 1H), 2.55-2.45 (m, 4 H), 2.1-2.0 (m, 2 H), 1.95-1.90 (t, J = 12 Hz, 2 H), 0.85 (s, 3 H), 0.75 (d, J = 6.40 Hz, 3 H), 0.65 (s, 3 H). ES-MS m/z (% rel. int.) 621.3 (100) [M + OAc^-].





Benzyl 3α-pentynoate-12α-Boc-aminobutyrate-5β-cholan-24-oate. Compound **3** (300 mg, 0.534 mmol) was dissolved in 1 mL dry DCM. Boc-GABA-OH (162 mg, 0.8 mmol) in 0.4 mL dry DCM and DMAP (130 mg, 1.068 mmol) in 0.4 mL dry DCM. The reaction mixture was allowed to stir at 0°C under argon for 10 min after which EDC was added portion wise and the stirring was allowed to continue for another 30 minutes in an ice bath. Finally, the reaction mixture was allowed to warm slowly and was left to stir overnight at room temperature. The residue was purified by column chromatography (silica gel Hexane/EtOAc 3:1; Rf: 0.38) to give **4** as a yellow oil (40% yield). ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.38 − 7.36 (m, 5 H), 5.15 (m, 1H 12β), 5.1 (d, J = 1.8 Hz, 2 H), 4.75 (br, m, 1H 3β), 3.15 (br m, 2H),2.60-2.50 (m, 2H), 2.55-2.45 (m, 4 H), 2.25 (br m, 2H), 2.1-2.0 (m, 2 H), 1.95-1.90 (t, J = 12 Hz, 2 H), 0.85 (s, 3 H), 0.75 (d, J = 6.40 Hz, 3 H), 0.65 (s, 3 H). ES-MS m/z (% rel. int.) 806.5 (100) [M+ $OAc]^-$.

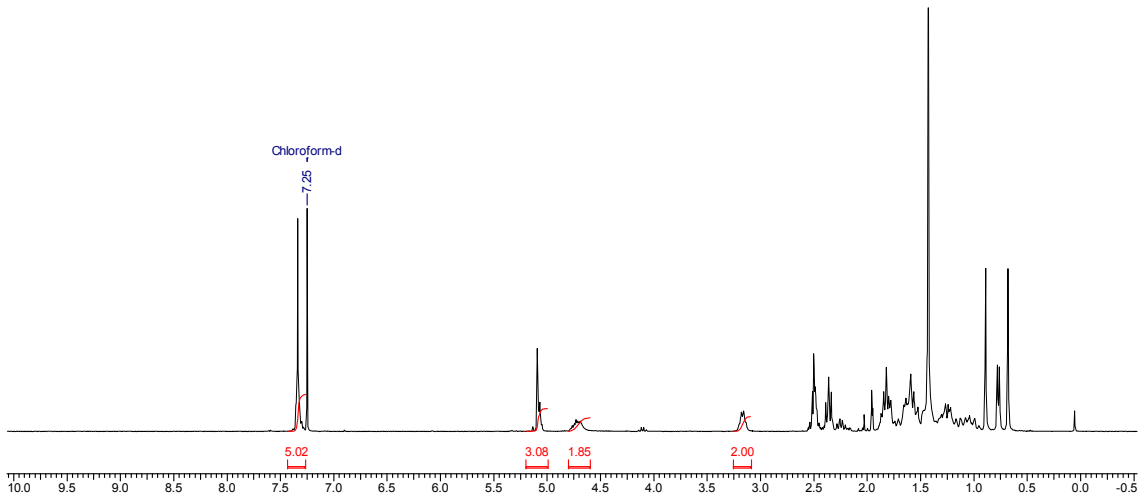


3

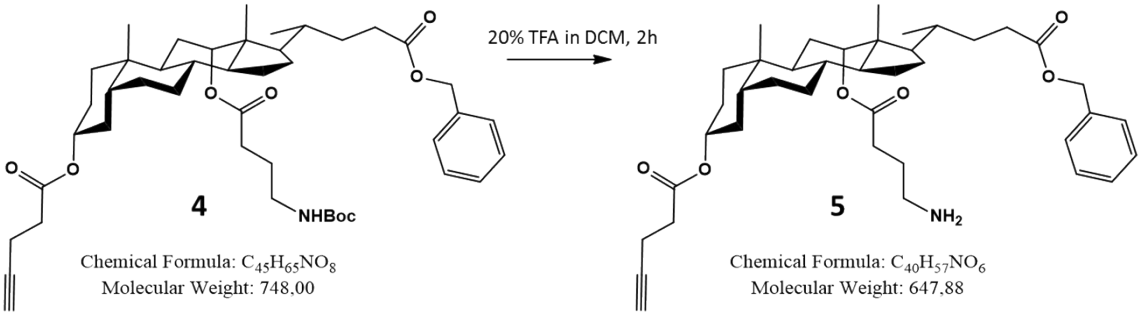
Chemical Formula: $C_{36}H_{50}O_5$
Molecular Weight: 562,78

4

Chemical Formula: $C_{45}H_{65}NO_8$
Molecular Weight: 748,00



Boc deprotection. Compound **4** (374.41 mg, 0.501 mmol) was dissolved in 4 mL DCM and 0.8 mL TFA was added. The reaction was stirred for 2h at room temperature. Then the solvent was evaporated and no further purification was needed (R_f: 0.13). 99% yield. 1H-NMR (300 MHz, $CDCl_3$); δ 7.38 − 7.36 (m, 5 H), 5.15 (m, 1H 12β), 5.1 (d, J = 1.8 Hz, 2 H), 4.75 (br, m, 1H 3β), 3.15 (br m, 2H),2.60-2.50 (m, 2H), 2.55-2.45 (m, 4 H), 2.25 (br m, 2H), 2.1-2.0 (m, 2 H), 1.95-1.90 (t, J = 12 Hz, 2 H), 0.85 (s, 3 H), 0.75 (d, J = 6.40 Hz, 3 H), 0.65 (s, 3 H). ES-MS m/z (% rel. int.) 648.4 (100) [M+ H^+].

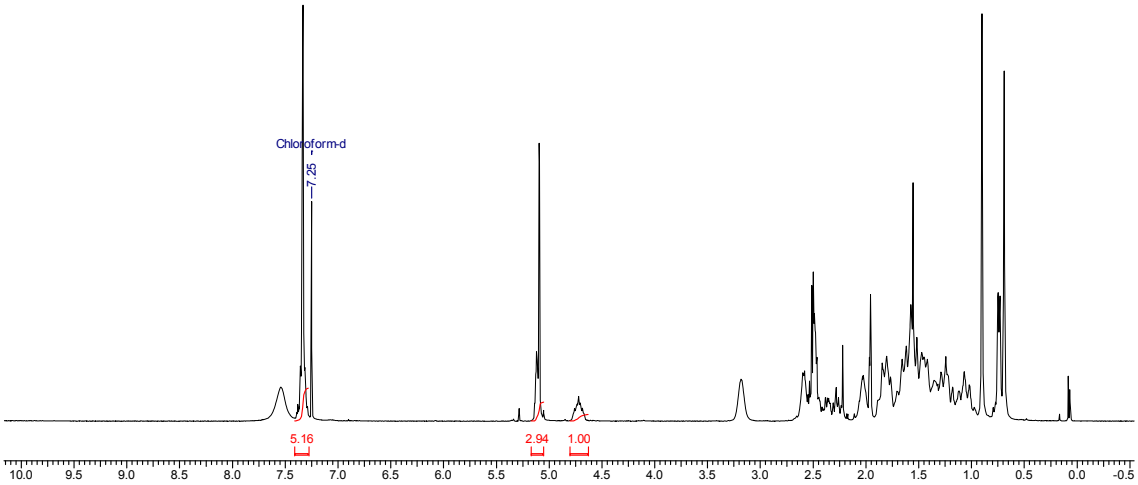


4

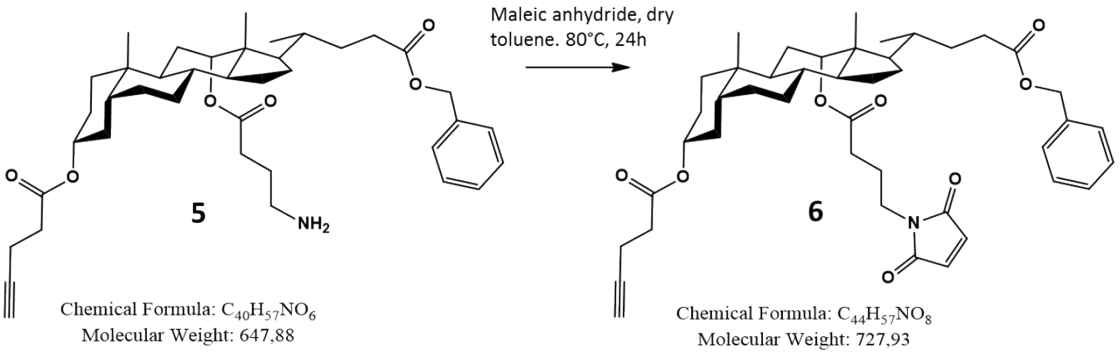
Chemical Formula: $C_{45}H_{65}NO_8$
Molecular Weight: 748,00

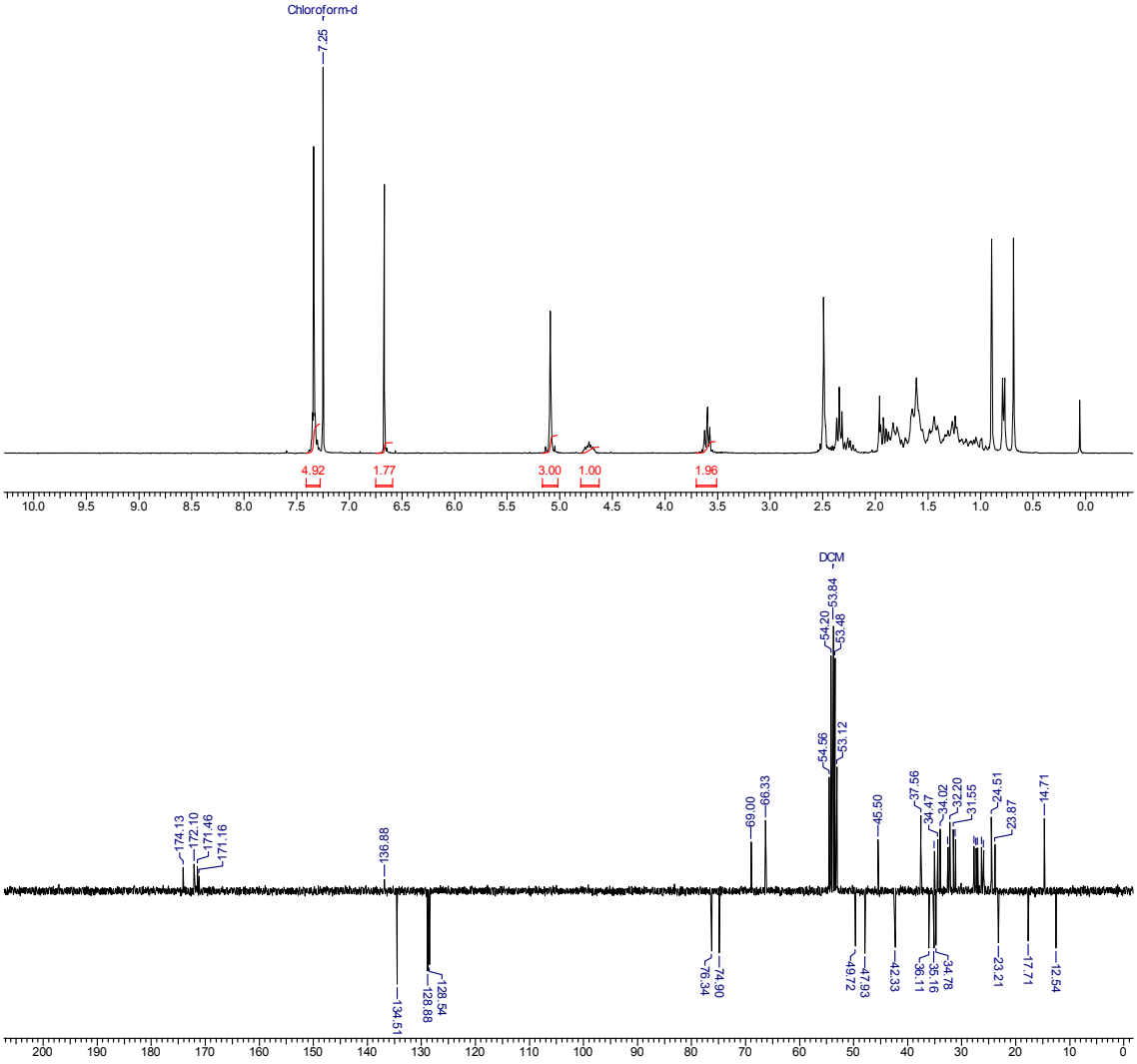
5

Chemical Formula: $C_{40}H_{57}NO_6$
Molecular Weight: 647,88



Benzyl 3α-pentynoate-12α-4-maleimido-butyrate-5β-cholan-24-oate. Compound **5** (107.6 mg, 0.166 mmol) was dissolved in 2mL dry toluene. Maleic anhydride (32.56 mg, 0.332 mmol) is added to the reaction in 2 mL dry toluene. The reaction is refluxed at 80°C for 24h. The solvent was evaporated and the residue was purified by column chromatography (fine silica gel Hexane/EtOAc 4:1; Rf: 0.2) to give **6** as a brownish oil (38% yield). 1H-NMR (300 MHz, $CDCl_3$); δ 7.38 – 7.36 (m, 5 H), 6.65 (s, 2H), 5.15 (m, 1H 12β), 5.1 (d, J = 1.8 Hz, 2 H), 4.75 (br, m, 1H 3β), 3.55 (t, 2H),2.55-2.50 (m, 2H), 2.45 (m, 4 H), 2.25 (br m, 2H), 1.95-1.90 (t, J = 12 Hz, 2 H), 0.85 (s, 3 H), 0.75 (d, J = 6.40 Hz, 3 H), 0.65 (s, 3 H).). 13C NMR (500 MHz, $CDCl_3$): δ = 174.13 (COOR-C24), 172.10 (2C maleimides), 171.46 (COOR-C3), 171.16 (COOR-C12), 136.88 (C - benzyl group), 134.51 (2CH maleimides), 128.88 (2CH-benzyl ring), 128.54 (2CH- benzyl ring), 128.20 (CH-benzyl ring), 76.34 (CH ester), 74.90 (CH ester), 69.00 (C-Alkyne), 66.33 (CH2-benzyl ester), 49.72 (CH), 47.93 (CH), 45.50 (C), 42.33 (CH), 37.56 (CH_2), 36.11 (CH), 35.16 (CH), 34.78 (CH), 34.47 (CH_2), 34.02 (C), 32.50 (CH_2), 32.20 (CH_2), 31.55 (CH_2), 31.20 (CH_2), 26.80 (CH_2), 26.65 (CH_2), 26,10 (CH_2), 25.05 (CH_2), 25.00 (CH_2), 24.51 (CH_2), 23.87 (CH_2), 23.21 (19-CH_3), 17.71 (21 – CH_3), 14.71 (CH_2), 12.54 (18-CH_3). ES-MS m/z (% rel. int.) 745.4 (100) [M+ NH_4^+]. HR-MS (ES) m/z calcd. for $C_{44}H_{57}NO_8$ + NH_4^+ 727.40842, found 745.4487.

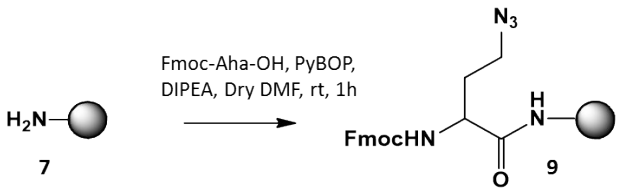




3. Peptide synthesis:

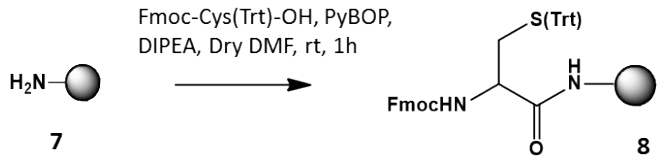
3. I. Manual coupling protocols

Immobilization of Fmoc-Aha-OH on Rink amide ChemMatrix resin



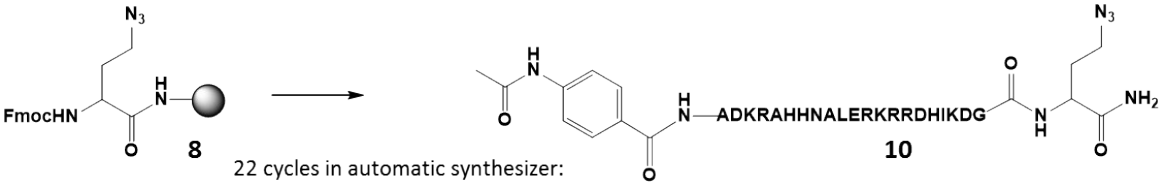
To a suspension of resin (200 mg, 0.54 mmol/g) in DMF (dry, 10 mL/g resin), were added Fmoc-Aha-OH (118.7 mg, 0.324 mmol), HBTU (122.87 mg, 0.324 mmol) and DIPEA (0.112 mL, 0.648 mmol). The mixture was shaken at room temperature for 1 h. The resin was then capped with acetic anhydride (0.061 mL, 0.648 mmol) and DIPEA (0.112 mL, 0.648 mmol) in dry DMF (3 mL) 2 times for 30 min.

Immobilization of Fmoc-Cys(Trt)-OH on Rink amide ChemMatrix resin

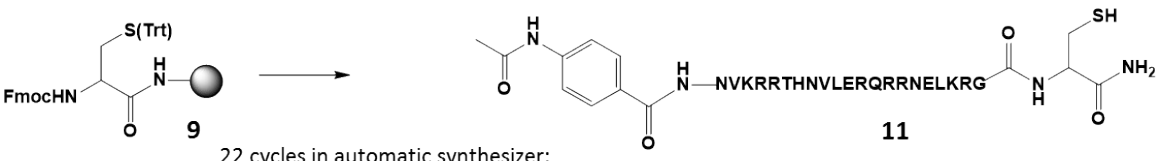


At a suspension of resin (200 mg, 0.54 mmol/g) in DMF (dry, 10 mL/g resin), were added Fmoc-Cys(Trt)-OH (189.7 mg, 0.324 mmol), HBTU (122.9 mg, 0.324 mmol) and DIEA (0.112 mL, 0.648 mmol). The mixture was shaken at room temperature for 1 h. After the reaction, the resin was washed with DMF, ACN and MeOH. The resin was then capped with acetic anhydride (0.061 mL, 0.648 mmol) and DIPEA (0.112 mL, 0.648 mmol) in dry DMF (3 mL) 2 times for 30 min.

3. II. Automated synthesis of linear peptides



Chemical Formula: $C_{117}H_{189}N_{49}O_{33}$
Exact Mass: 2808.46
Molecular Weight: 2810.06



Chemical Formula: $C_{121}H_{208}N_{50}O_{33}S$
Exact Mass: 2921.58556
Molecular Weight: 2923.32642

Automated solid phase peptide synthesis was carried out on a Syro synthesizer from Biotage using standard Fmoc/tBu chemistry with HBTU as coupling reagent and 20% piperidine in DMF as deprotection reagent.

Fmoc protected resin (0.025 mmol) was subjected to automated synthesis where solutions of Fmoc-N_{σ}-protected amino acids (0.5 M in DMF) were prepared. Each reaction coupling lasts 1h (amino acids 0.250 mmol; HBTU 0.250 mmol in DMF, 0.5 M; DIPEA 0.250 mmol 2 M) and followed by Fmoc deprotection with 40% piperidine in DMF. After each reaction the resin was washed with DMF (9 x). The peptide was then cleaved for the resin and deprotected with a cocktail of TFA/TIS/water = 95: 2.5: 2.5 for 4h. After precipitation in cold ether, the peptide was analyzed by RP-HPLC and MALDI.

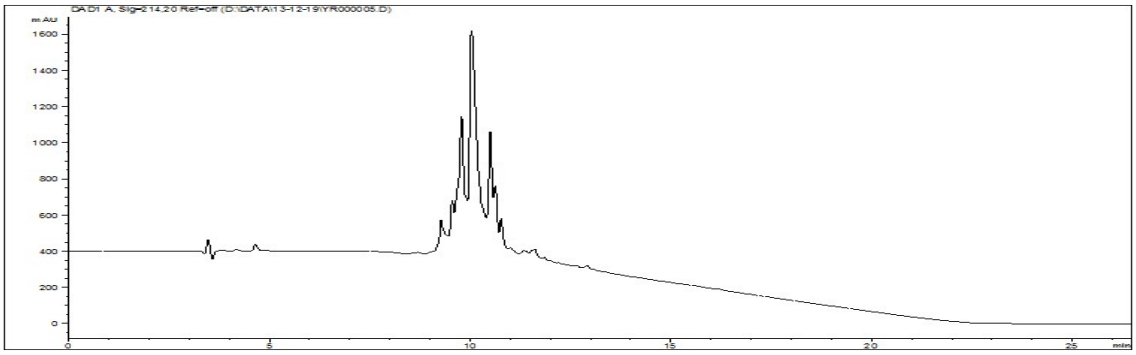


Figure S1: RP-HPLC Chromatogram of crude compound **10** (C4, 300Å column using a gradient from 0 to 100 % CH_3CN in 15 minutes)

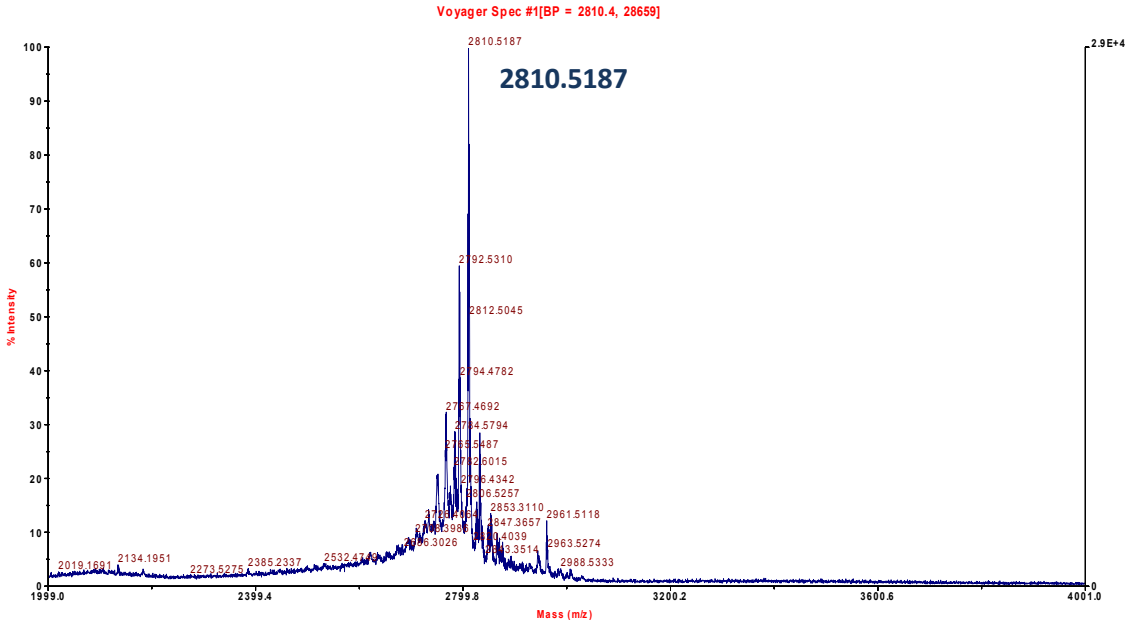


Figure S2: MALDI-TOF Spectrum of compound **10** from peak at t = 10.034 min. E.M. calcd. for $C_{117}H_{189}N_{49}O_{33}$ = 2808.46. Found M + H^+ = 2810.5187.

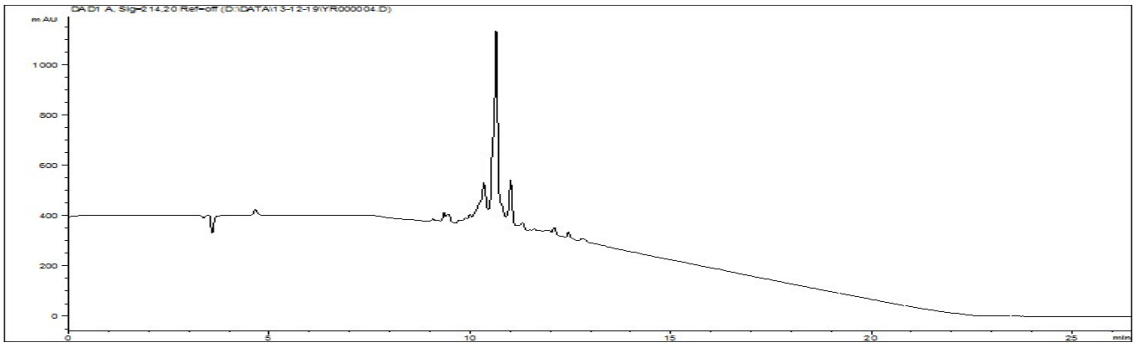


Figure S3: RP-HPLC chromatogram of crude compound **11** (C4, 300Å column using a gradient from 0 to 100 % CH_3CN in 15 minutes)

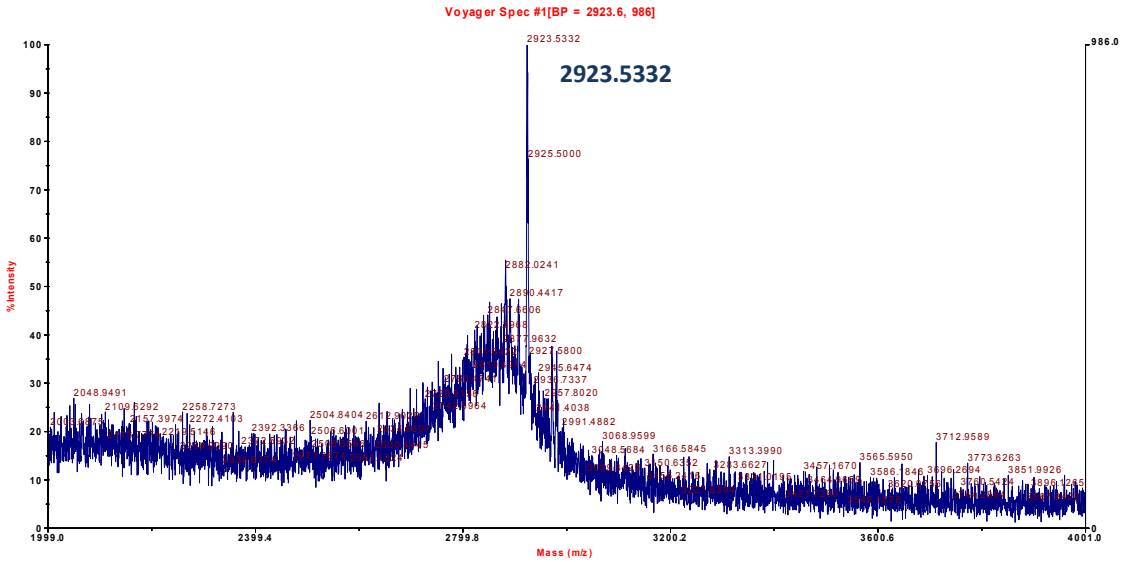
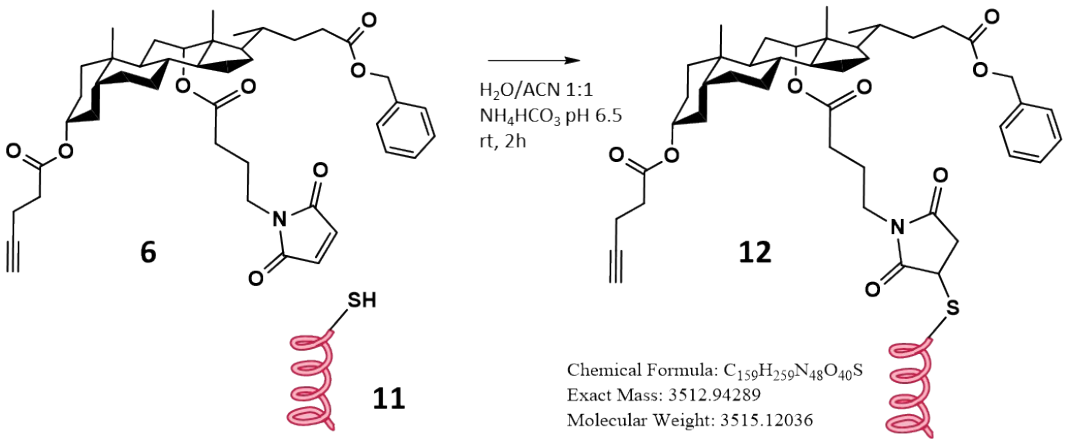


Figure S4: MALDI-TOF Spectrum of crude compound **11** from peak at t = 10.651 min. E.M. calcd for $C_{121}H_{208}N_{50}O_{33}S$ = 2921.59. Found: 2923.5332 = M + H^+.

4. Conjugation of peptides to the steroid scaffold

Conjugation of peptide cMyc to C12 position of scaffold

Scaffold 6 (1.24 mg, 0.002 mmol) and peptide 11 (8 mg, 0.003 mmol) were dissolved in 1 mL of a mixture H_2O/ACN (4:1). The pH was adjusted to 6.5 with NH_4HCO_3 buffer. The reaction was stirred for 2h and it was monitored by MALDI and HPLC. The solvent was evaporated and the crude compound 12 was subjected for CuAAC without prior purification.



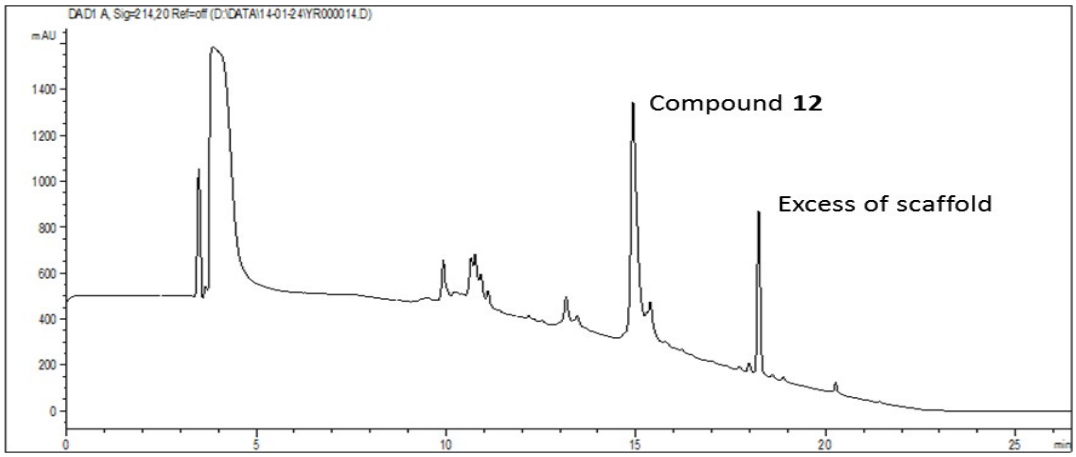
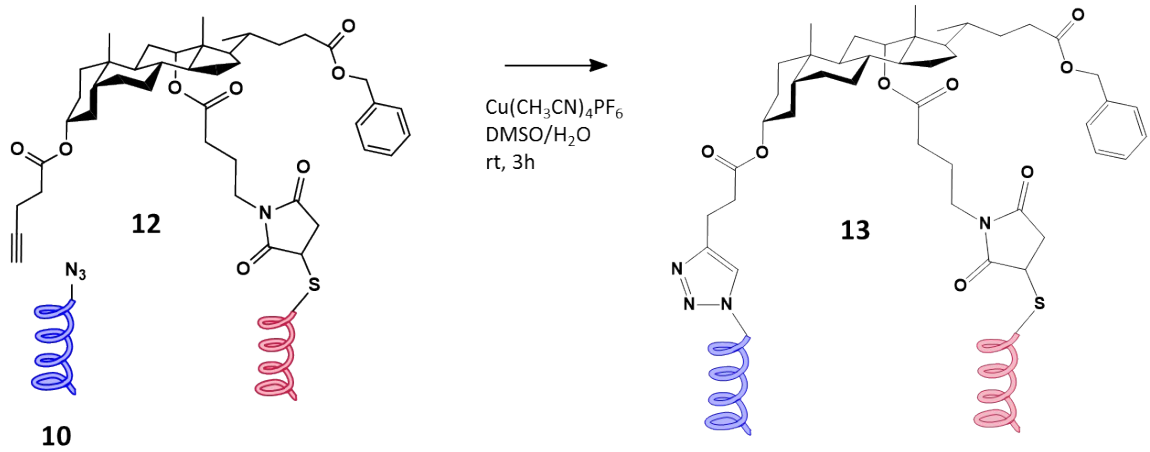


Figure S5: RP-HPLC Chromatogram of reaction mixture for synthesis of **12** after t = 2 h (C4, 300Å column using a gradient from 0 to 100 % CH_3CN in 15 minutes)

*Conjugation of peptide Max to C3 position of compound **12***

Compound **12** (5 mg, 0.0013 mmol) was dissolved in 0.2 mL dry DMSO. Peptide 10 (6.15 mg, 0.0022 mmol) in 0.1 mL H_2O/ACN 4:1 was added to the scaffold and the reaction was stirred for 10 min under argon. $Cu(CH_3CN)_4PF_6$ (5.1 mg, 0.014 mmol) dissolved in 0.075 mL dry DMSO was added to the reaction mixture. The reaction was monitored by HPLC and it was completed after 3h. Compound **13** was purified by RP-HPLC (yield of both conjugations: 24%).



Chemical Formula: $C_{282}H_{454}N_{100}O_{74}S$
Exact Mass: 6457.45572
Molecular Weight: 6461.31276

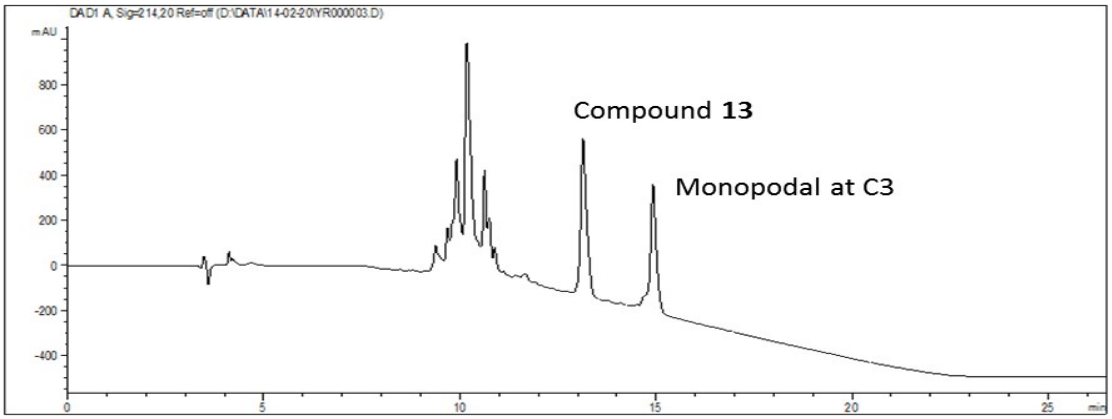


Figure S6: RP-HPLC Chromatogram of reaction mixture for synthesis of **13** at t = 3 h (C4, 300Å column using a gradient from 0 to 100 % CH_3CN in 15 minutes)

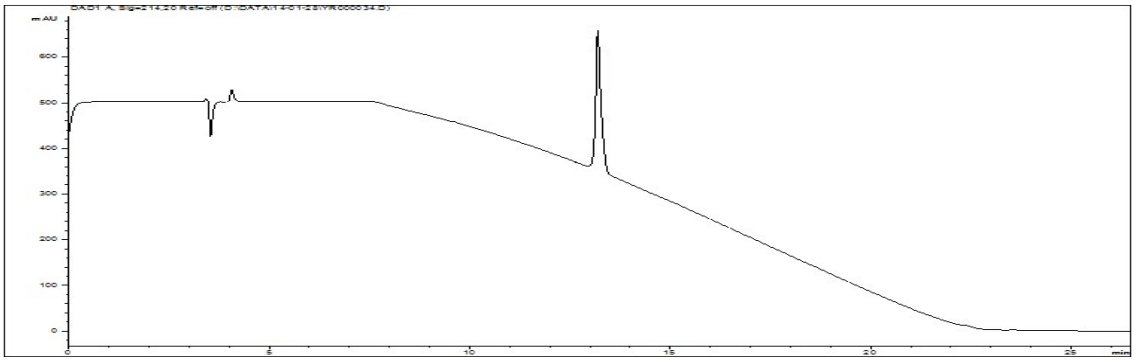


Figure S7: RP-HPLC Chromatogram of RP-HPLC purified compound **13** (C4, 300Å column using a gradient from 0 to 100 % CH_3CN in 15 minutes)

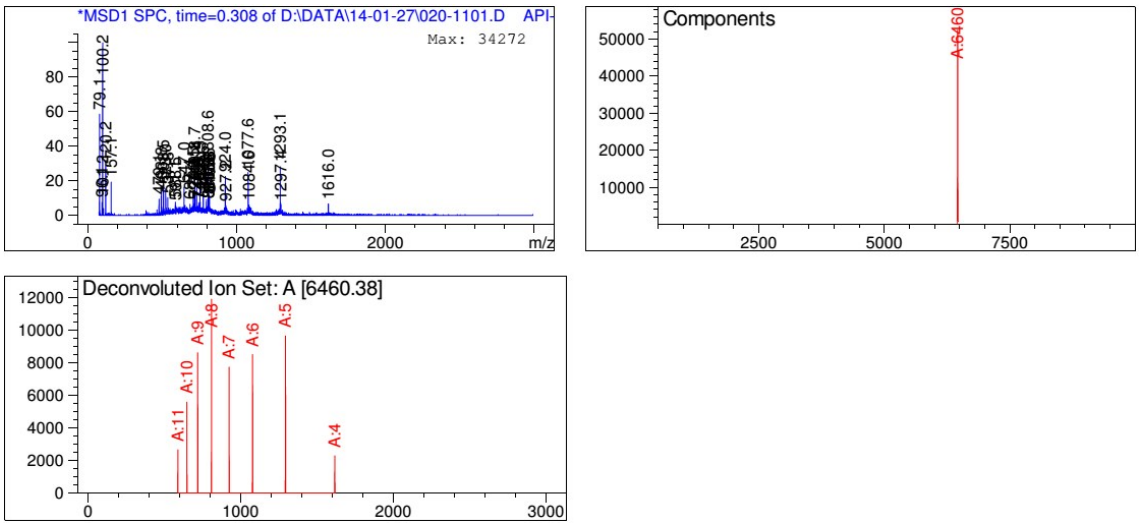


Figure S8: ESI-MS of RP-HPLC purified compound **13**. E.M. calcd. for $C_{282}H_{454}N_{100}O_{74}S$ = 6457.46 and deconvoluted mass found 6460.38

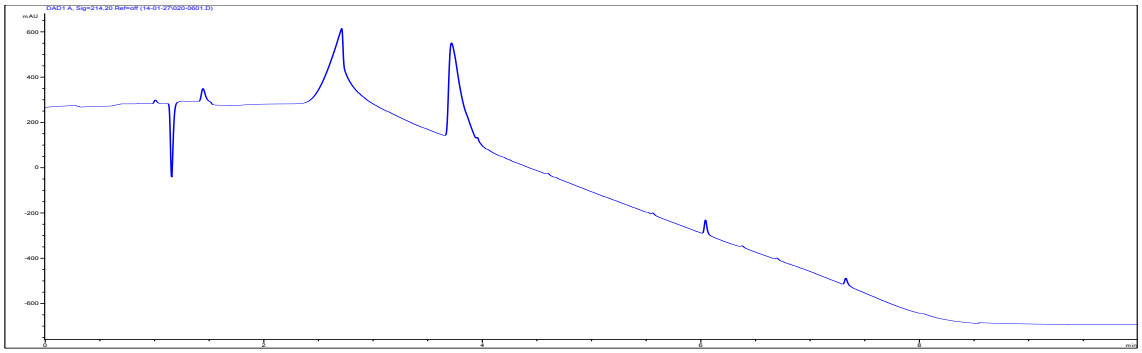


Figure S9: RP-HPLC trace from LC-MS for HPLC purified compound **13** (0-100% ACN in 6 min on Kinetex C18 100 Å, 150 x 2.1 mm, 2.6 µm, at 35 °C)

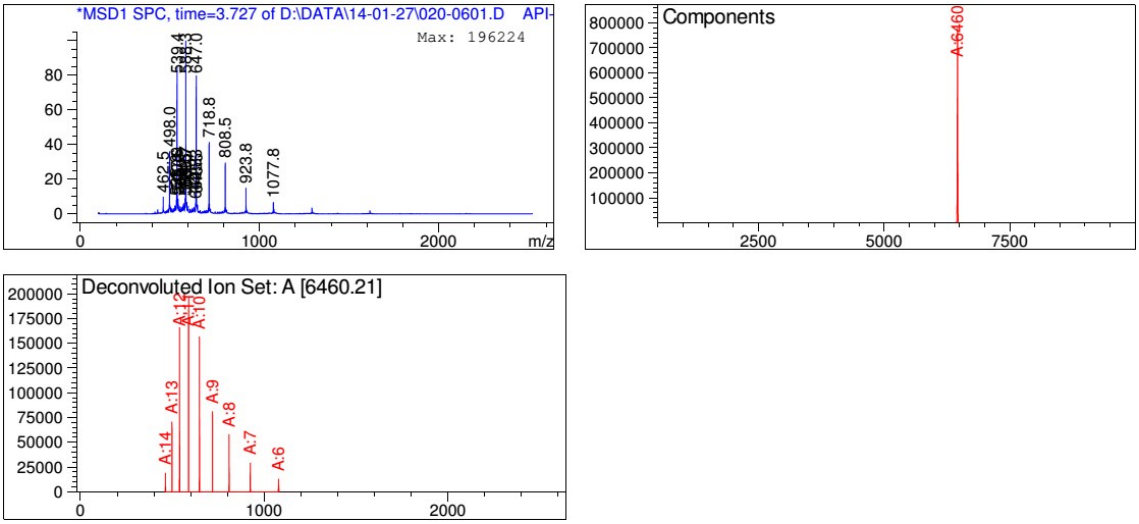


Figure S10: ESI-MS from LC-MS at r.t. = 3.727 min. E.M. calcd. for $C_{282}H_{454}N_{100}O_{74}S$ = 6457.46 and deconvoluted mass found 6460.21

5. ELECTROPHORETIC MOBILITY SHIFT ASSAY (EMSA)

5.I EMSA WITH UNLABELED DNA

Sample preparation:

The following stock solutions were prepared (fresh each time, except for DNA and peptide):

DNA: 1.67 µM prepared from E-Box (5' – CTA CTA GCA CGT GCT AGT AG – 3') & E-Box complement (5' – CTA CTA GCA CGT GCT AGT AG – 3') and Random (5' – GCG CGA GAA GGA AAG AAA GCC GG – 3') & complement (5' – CCG GCT TTC TTT CCT TCT CGC GC – 3') DNA solutions (obtained from Eurogentec purified over gold cartridge) by diluting with 20 µL 0.5 M Tris, pH = 8, 40 µL 2.5 M NaCl, 40 µL 0.025 M EDTA and then adding miliQ water such that the total volume is 1 mL. DNA was annealed by heating from room temperature to 95°C for 24 min in a Thermomixer. The machine was then turned off and the sample was allowed to cool down slowly.

Loading buffer: 20 µL Tris 1 M, pH = 7.6, 20 µL KCl 0.2 M, 20 µL $MgCl_2$ 0.1 M, 40 µL EDTA 0.025 M.

Sucrose: 30% sucrose in mQ (300 mg/mL)

Peptides: 10 µL stock solutions (10x) were prepared in miliQ water. Concentrations in lanes 1-10 in µM: (0, 1.67, 3.34, 5.01, 5.84, 6.68, 7.51, 8.35, 11.69, 16.7).

Loading mixture: The loading mixture comprised of: 10 µL mQ, 4 µL sucrose, 2 µL loading buffer, 2 µL DNA, 2 µL peptide. The loading mixture was prepared only 1-2 h prior to running of gels and kept on ice as soon as ready.

Preparation of Gels (for 2 Gels):

In a clean falcon tube the following were added (in given order): 15.6 mL mQ, 0.4 mL TBE, 4.0 mL of 40% acrylamide solution, 200 µL APS (10% w/w in mQ). The solution was mixed by sonication to remove any air bubbles and cooled to 0°C (1 h under ice). 20 µL of TEMED was then added to the mixture and was again mixed properly before pouring it gently along parallel glass plates. The glass plates were tapped gently to ensure removal of all air bubbles and the markers were squeezed between the plates to ensure uniform width of each well. Sufficient time was given for polymerization (~1 h).

Gel Electrophoresis:

A pre-run of the gels was performed prior to loading them. Care was taken to see that the gels were properly immersed in 0.2x TBE buffer (non-denaturing gel, without urea) and the loading wells were free from any air bubbles. Instrument settings: 150 V, 100 mA, 19 W for 30 mins at 4°C. The wells were washed after the pre-run. 5 µL of the loading mixture was then loaded onto the wells. Instrument settings: 150 V, 100 mA, 19 W for 45 mins at 4°C.

Staining of gels:

After the run, the gels were removed from the glass and were stained using 100 mL of 0.2x TBE buffer + 10 µL SybrGold® (Life Technologies™) stock solution 10,000X in DMSO. The gels were then washed twice with mQ and gently placed under a UV lamp (dark room) to observe the gel pattern. The presence of two bands at the dsDNA lane (figure **S12**) is consequence of the incomplete annealing of both DNA sequences. The lowest band corresponds to the ssDNA while the upshifted band is the dsDNA.

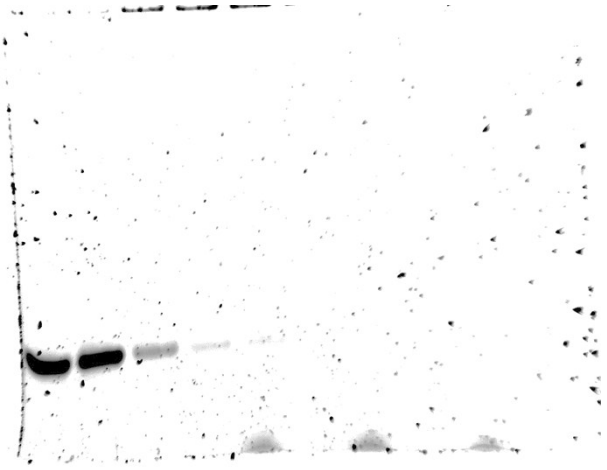


Figure S11: EMSA titration of the dipodal peptidosteroid **13** to the ramdom dsDNA stained with SybrGold.

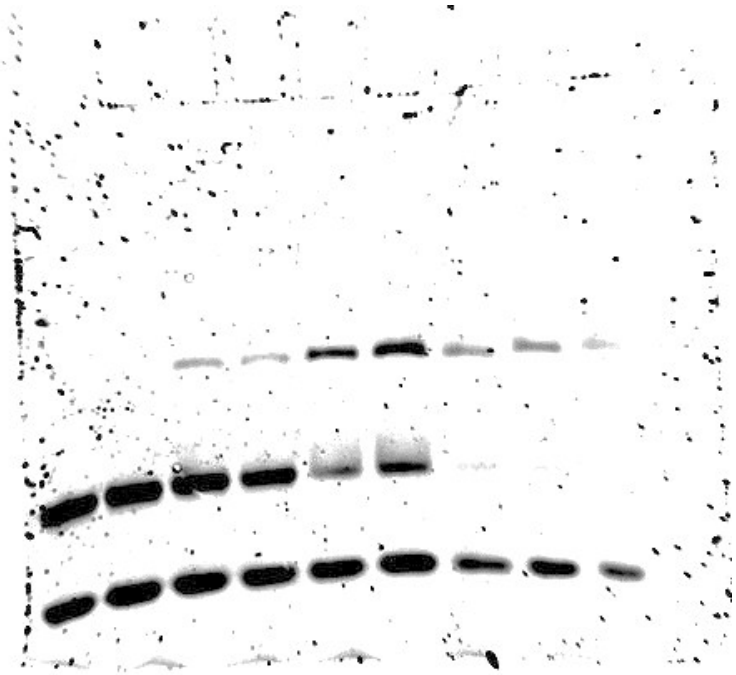


Figure S12. EMSA titration of the dipodal peptidosteroid **13** to the E-box binding site stained with SybrGold.

5.II EMSA WITH RADIOLABELED DNA

Preparation of ^{32}P-labeled dsDNA target

Oligonucleotide target sequence containing E-box binding site (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') was 5'-labeled using [γ-32P] ATP and T4 polynucleotide kinase (Fermentas) according to the manufacturer's protocol, and then purified using QIAquick Nucleotide Removal Kit (Qiagen). The 5'-end labeled

pyrimidine oligonucleotide was annealed with the unlabeled complementary strand. An amount of 5 nM dsDNA was prepared diluting 20 µL 0.5 M Tris, pH = 8, 40 µL 2.5 M NaCl, 40 µL 0.025 M EDTA and then adding milliQ water such that the total volume is 1 mL. The DNA was annealed in a heat block by heating from 95°C during 5 minutes followed by slow cooling to room temperature. As competitor DNA the following sequences were used: (i) 45-mer double strand (dsDNA) 5'-AGCAGAGGGCGTGGGGGAAAAGAAAAAAGATCCACCGGTCGCCAC-3' and its complementary strand. (ii) a 15-mer single strand (ssDNA) 5'-CCTTTTCTTTTTTCT-3'.

Loading buffer: 20 µL Tris 1 M, pH = 7.6, 20 µL KCl 0.2 M, 20 µL $MgCl_2$ 0.1 M, 40 µL EDTA 0.025 M.

Sucrose: 30% sucrose in mQ (300 mg/mL)

Peptide **13**: 10 µL stock solutions (10x) were prepared in MiliQ water (0, 1.25, 2.5, 5, 10, 20 µM), and (0, 0.312, 0.625, 1.25, 2.5, 5, 10, 20 µM).

Loading mixture: The loading mixture comprised of: 10 µL mQ, 4 µL sucrose, 2 µL loading buffer, 2 µL DNA, 2 µL peptide. The loading mixture was prepared only 1 h prior to running of gels and kept on ice as soon as ready.

Preparation of Gels (for 1 Gel):

In a clean glass beaker the following reagents were added (in given order): 21.57 mL mQ, 0.6 mL TBE 10X, 7.5 mL of 40% acrylamide solution (29:1), 0.3 mL APS (10% w/w in mQ) and 30 µL of TEMED was then added to the mixture, and mixed properly before pouring it gently along parallel glass plates. The glass plates were tapped gently to ensure removal of all air bubbles and the markers were squeezed between the plates to ensure uniform width of each well.

Sufficient time was given for polymerization (40 minutes).

Electrophoresis:

A pre-run of the gels was performed prior to loading them. Care was taken to see that the gels were properly immersed in 0.5x TBE buffer (non-denaturing gel, without urea) and the loading wells were free from any air bubbles. The wells were washed after the pre-run. Instrument settings: 150 V, 100 mA, 19 W for 45 minutes with circulation water cooling. 5 µL of the loading mixture was then loaded onto the wells. Instrument settings: 150 V, 100 mA, 19 W for 45 minutes with circulation water-cooling. The gels were frozen and analyzed by phosphor imaging using Molecular Imager FX and the data were processed using Quantity One software (BioRad).

-EMSA with radiolabeled 50-mer dsDNA:

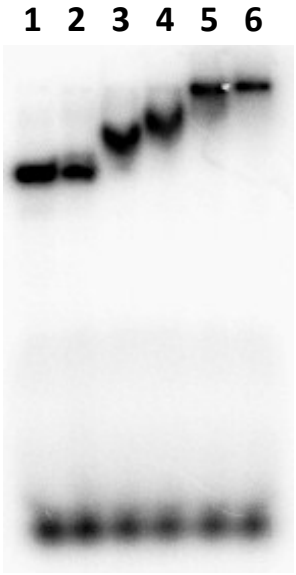


Figure S13. EMSA titration of the peptide-steroid conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') at 5 nM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.125, 0.25, 0.5, 1, and 2 µM of **13**.

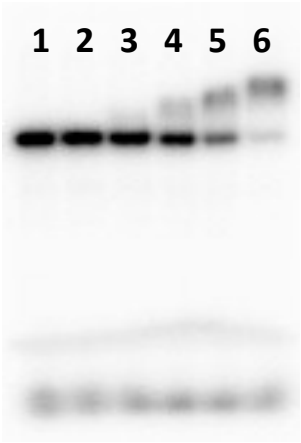


Figure S14. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') at 5 nM in the presence of competitor DNA sequence (5'-AGCAGAGGGCGTGGGGGAAAAGAAAAAAGATCCACCGGTCGCCAC-3') at 500 nM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.125, 0.25, 0.5, 1, and 2 µM of **13**.

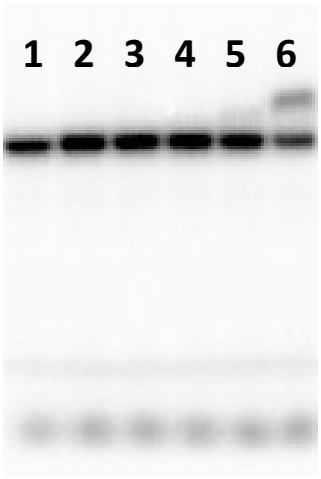


Figure S15. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') at 5 nM in the presence of competitor DNA sequence (5'-AGCAGAGGGCGTGGGGGAAAAGAAAAAAGATCCACCGGTCGCCAC-3') at 2000 nM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.125, 0.25, 0.5, 1, and 2 µM of **13**.

-EMSA with radiolabeled 26-mer dsDNA:

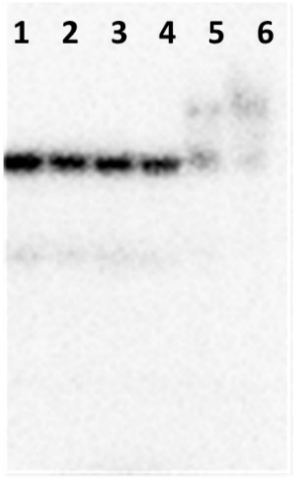


Figure S16. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' –TCGCTACTAGCACGTGCTAGTAGGTG – 3') at 5 nM: First lane in the gel: pyrimidine strand. Lanes 2-6 contain peptide concentrations of 0, 0.0312, 0.0625, 0.125 and 0.5 µM of **13**.

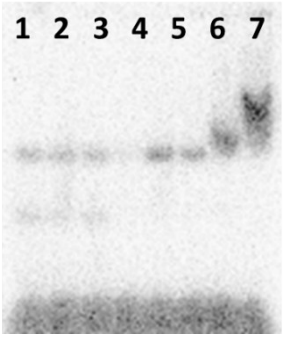


Figure S17. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' –TCGCTACTAGCACGTGCTAGTAGGTG – 3') at 5 nM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.0312, 0.0625, 0.125, 0.25, 0.5, 1 and 2 µM of **13**.

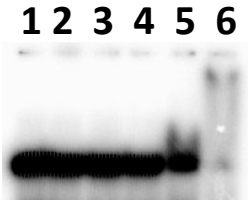


Figure S18. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') at 5 nM in the presence of competitor ssDNA sequence (5'-CCTTTTCTTTTTTCT-3') at 3 µM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.125, 0.25, 0.5, 1, and 2 µM of **13**.

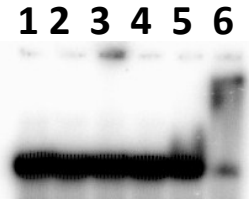


Figure S19. EMSA titration of the dipodal conjugate **13** to the 5'-labeled ^{32}P- E-box sequence (5' – CCATGGCGAGCGTCGCTACTAGCACGTGCTAGTAGGTGCGCTATCTAAGG – 3') at 5 nM in the presence of competitor ssDNA sequence (5'-CCTTTTCTTTTTTCT-3') at 12 µM: First lane in the gel: dsDNA. Lanes 2-6 contains peptide concentrations of 0.125, 0.25, 0.5, 1, and 2 µM of **13**.