Supporting Information for

Design, Synthesis and Biological Evaluation of a Cyclic Bile Acid-Peptide Conjugate: Towards the Conformational Mimicry of the Measles Virus HNE Loop

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I. Analytical data for synthesis of cyclic peptidosteroid 2

Unless otherwise stated, RP-HPLC analyses were performed on an Agilent 1100 Series instrument with a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 μm at 35 °C). A flow rate of 1 ml/min and with the following solvent systems: 0.1% TFA in H_2O (A) and MeCN (B). The column was flushed for 3 min with 100% A, then a gradient from 0 to 100% B over 15 min was used, followed by 5 min of flushing with 100% B. Alternatively, a Phenomenex Jupiter C4 column (250 x 4.6 mm, 5 μm at 35 °C) was used.

The identity of peaks was confirmed by MALDI-TOF analysis.

Synthesis of peptidosteroid 2: *Relevant chromatograms and MS spectra.*

Details about the synthesis have been described in the experimental section of the paper. After each step of the synthesis, a small amount of resin (typically <1 mg) was irradiated at 365 nm to cleave the compound for analysis.

In principle all routes depicted in scheme 4 can lead to the desired compound, as concluded from test reactions on small scale for all depicted intermediates. However, in order to obtain enough material the route **5 → 6 → 7 → 8 → 12 → 2** was chosen.

*Intermediate **5**:*

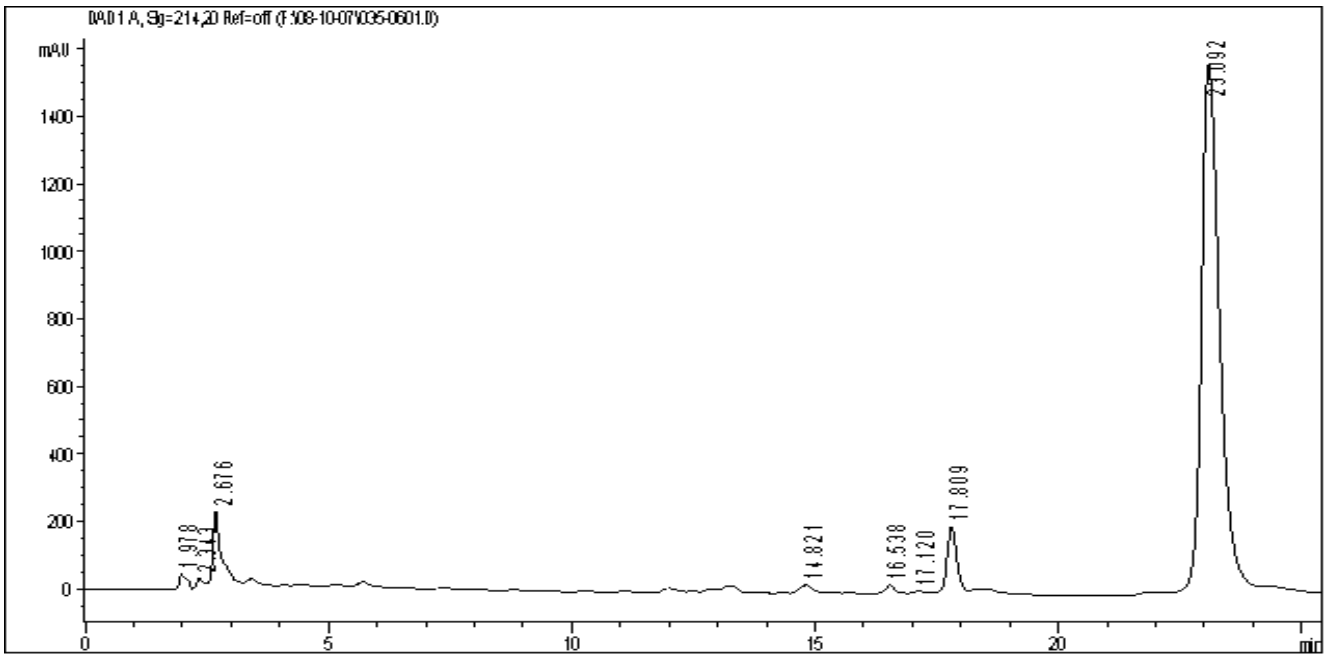


Figure 1a Crude HPLC chromatogram of **5** (Jupiter column) at 214 nm, intermediate **5** is eluting at t_r = 23.1 min

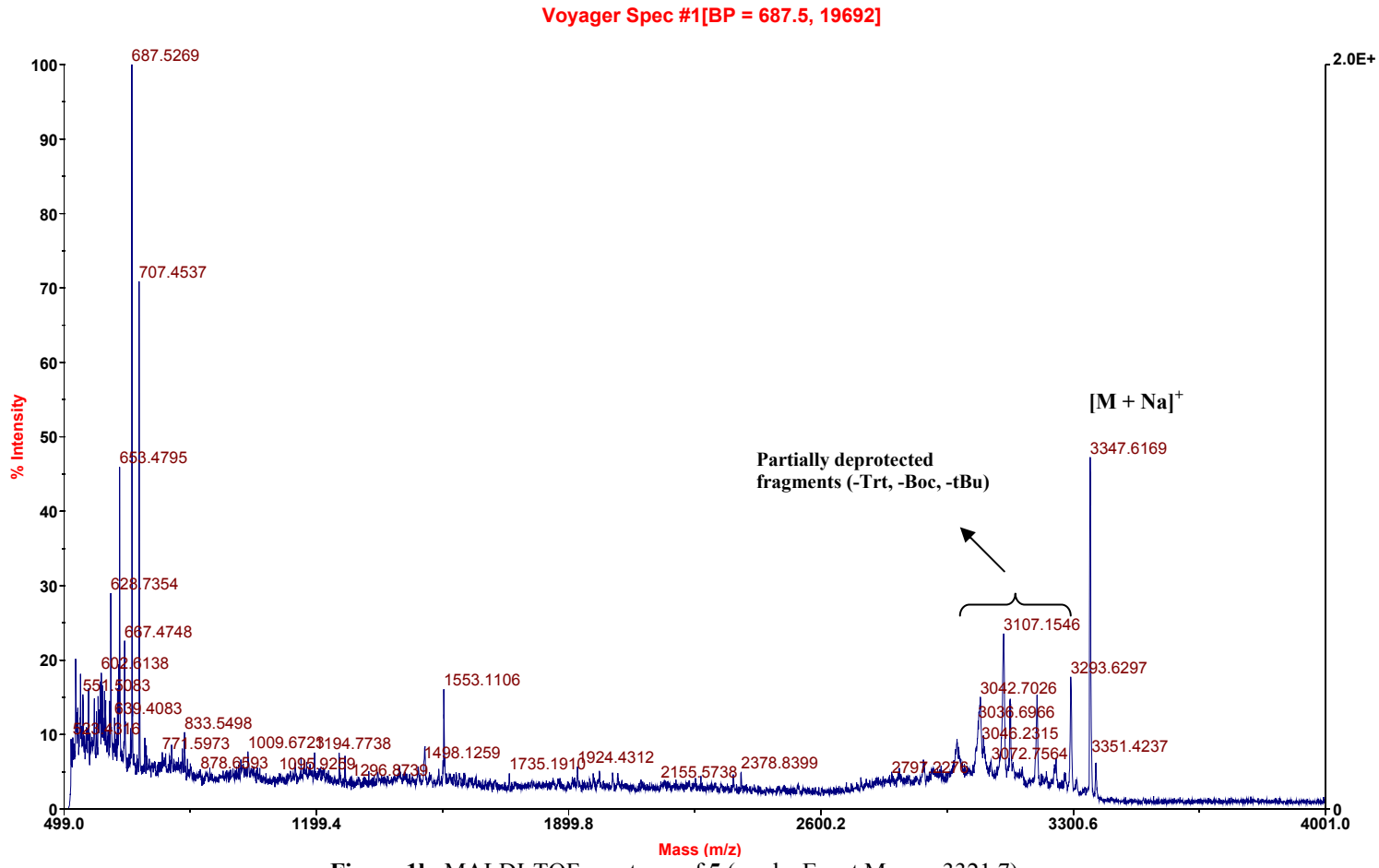


Figure 1b MALDI-TOF spectrum of **5** (crude, Exact Mass = 3321.7)

*Intermediate **6**:*

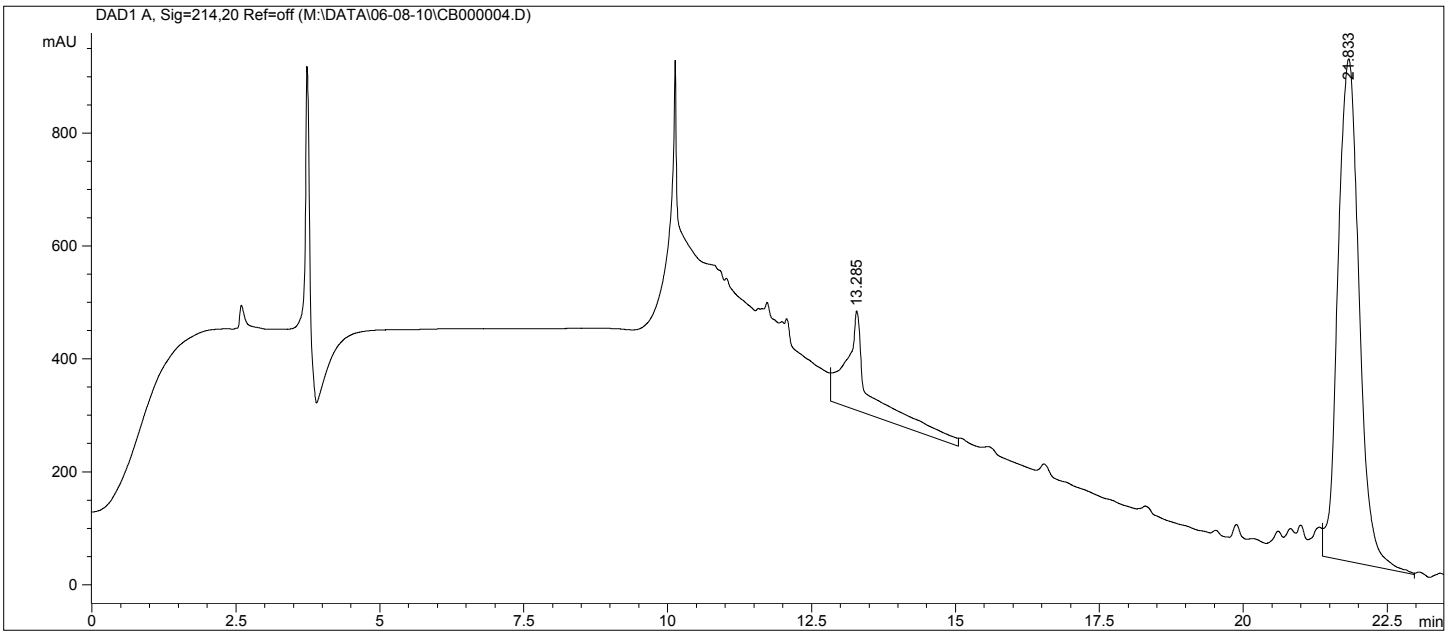


Figure 2a Crude HPLC chromatogram of **6** at 214 nm, intermediate **6** is eluting at t_r = 21.8 min

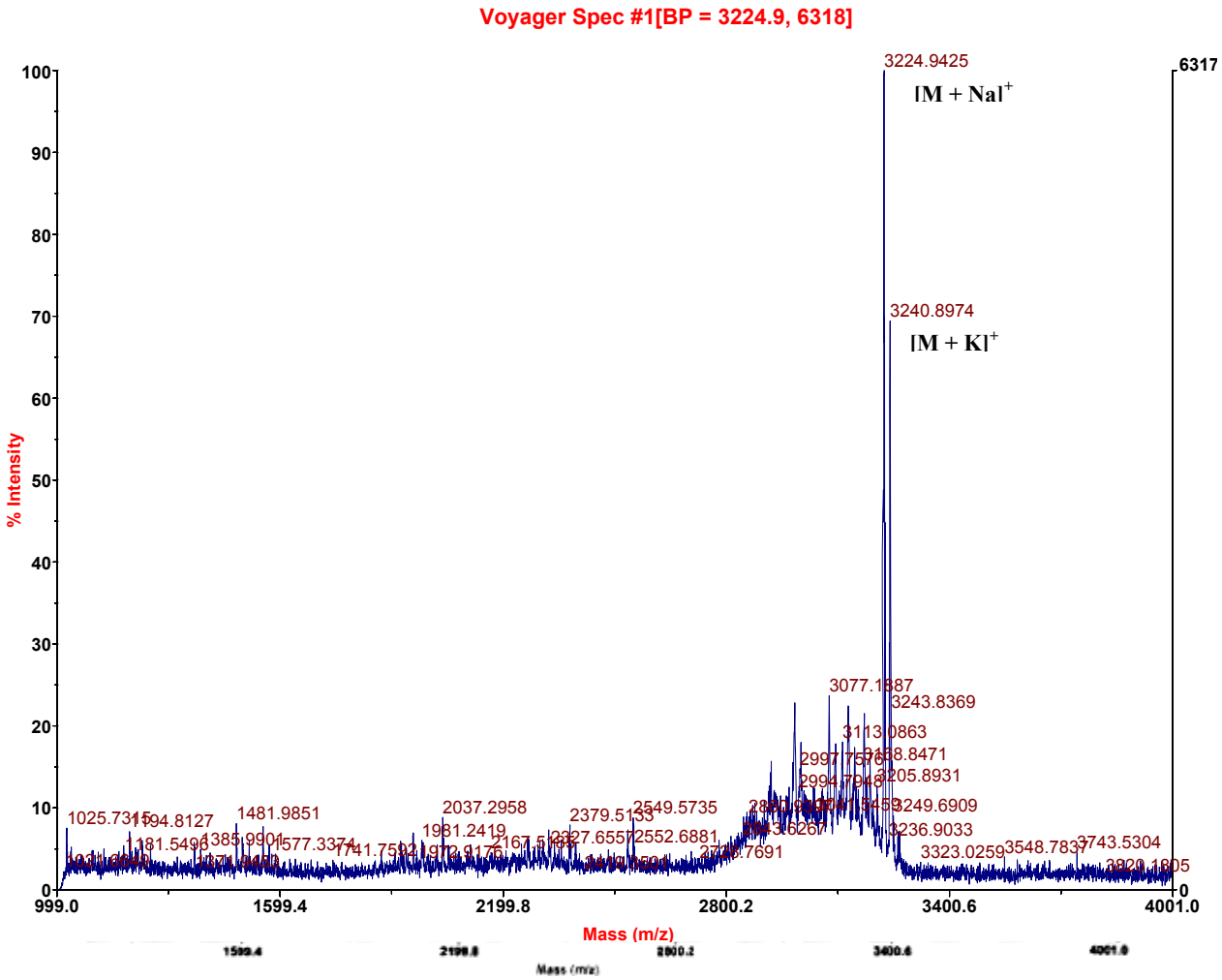


Figure 2b MALDI-TOF spectrum of 6 (crude, Exact Mass = 3200.7)

Intermediate 7:

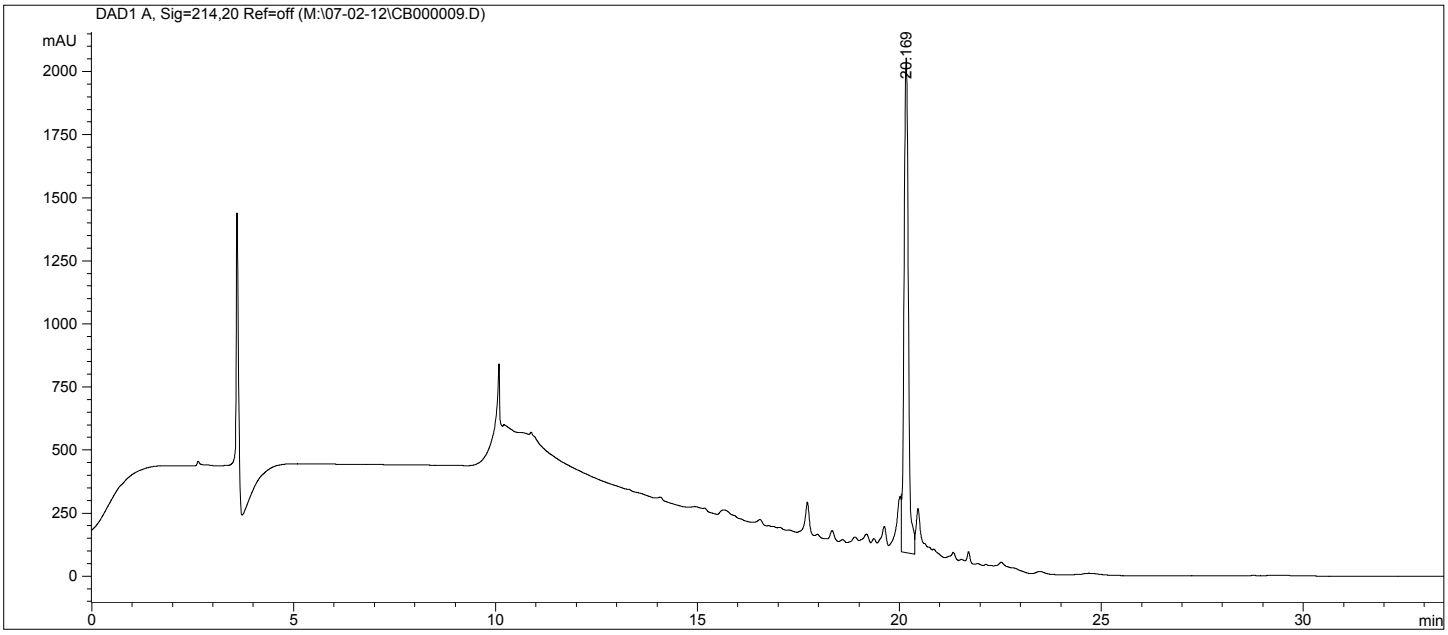


Figure 3a Crude HPLC chromatogram of **7** at 214 nm, intermediate **7** is eluting at t_r = 20.2 min

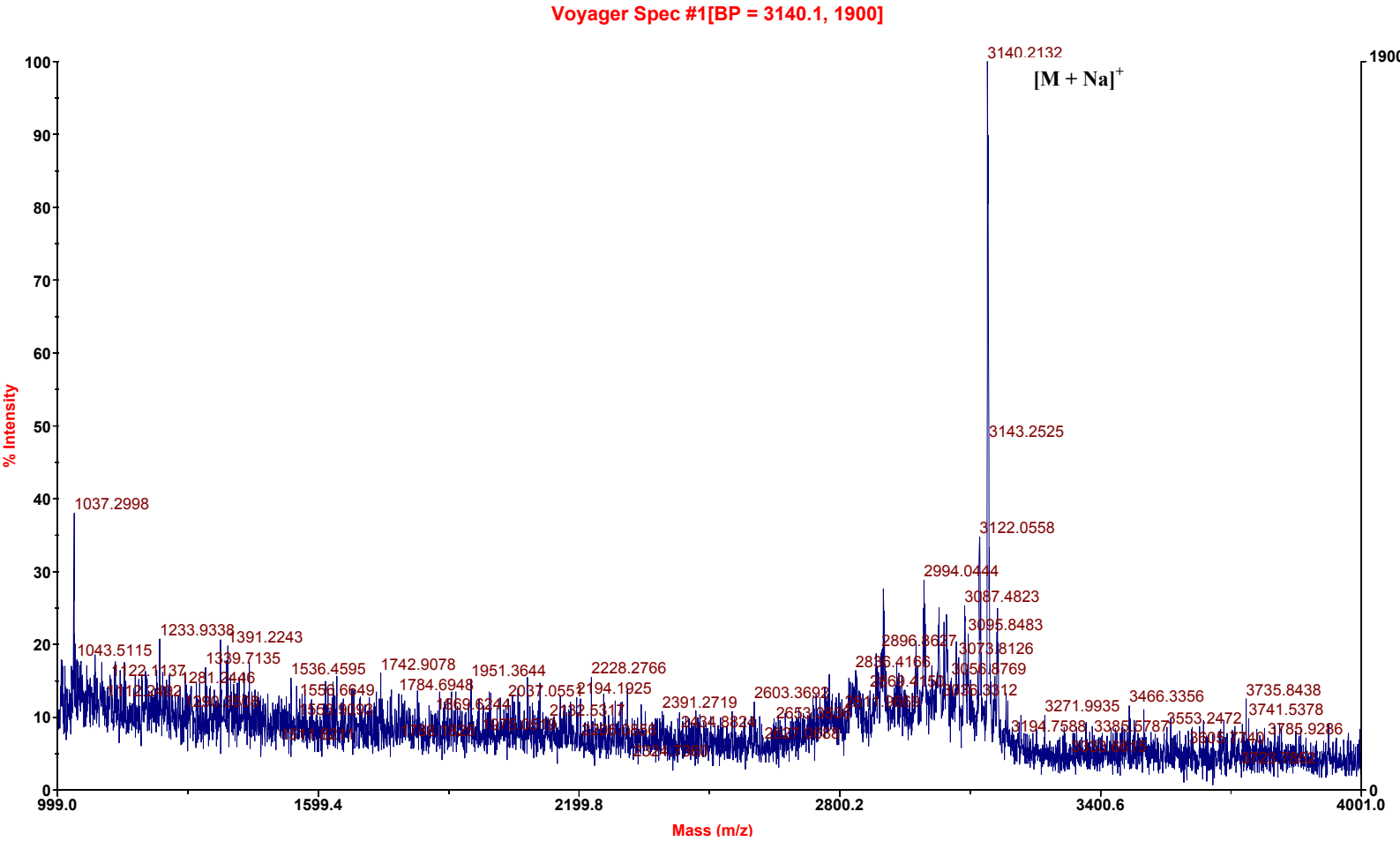


Figure 3b MALDI-TOF spectrum of **7** (crude, Exact Mass = 3115.6)

*Intermediate **8**:*

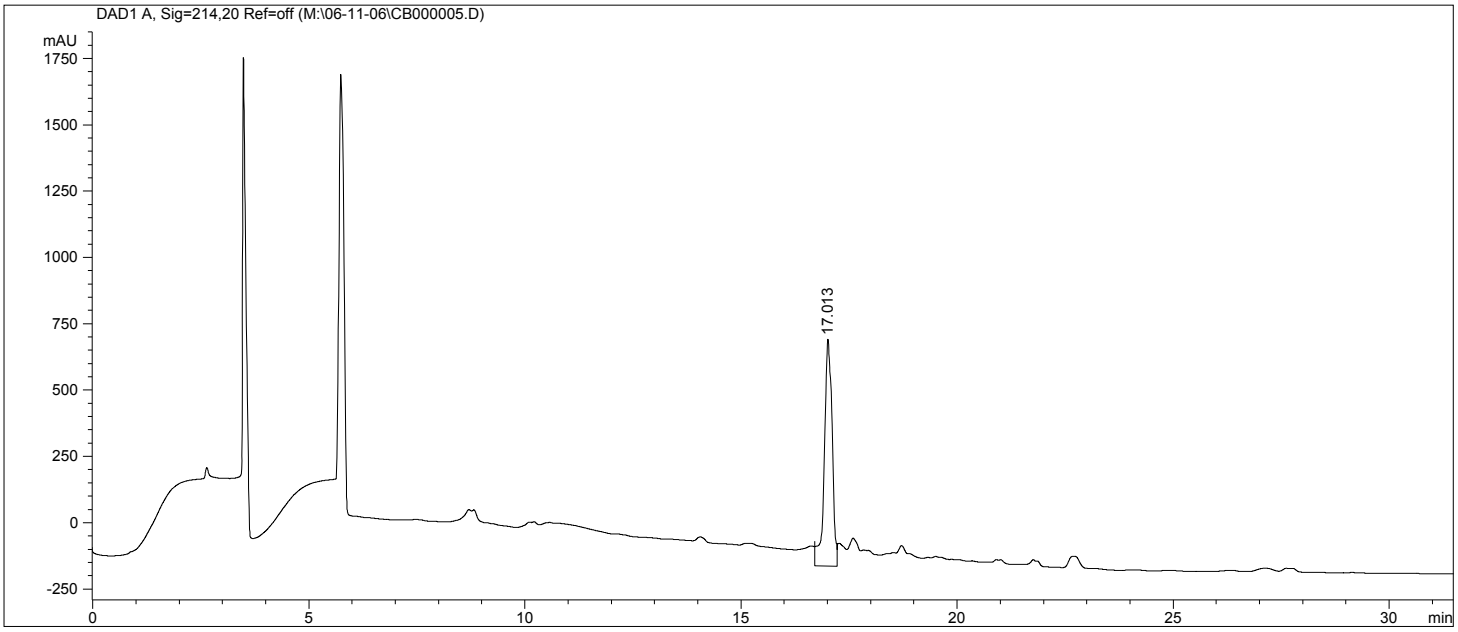


Figure 4a Crude HPLC chromatogram of **8** at 214 nm, intermediate **8** is eluting at t_r = 17.0 min

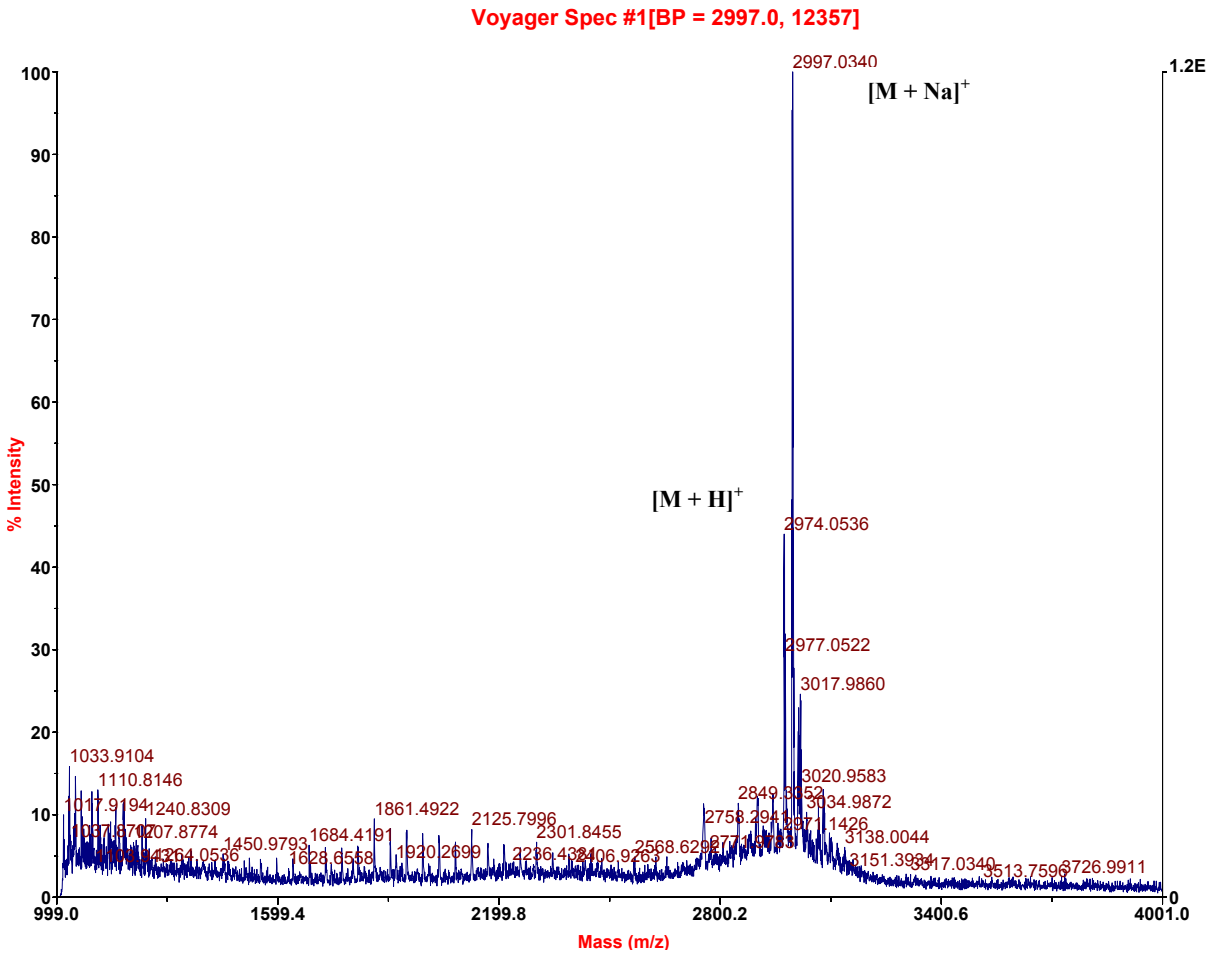


Figure 4b MALDI-TOF spectrum of **8** (crude, Exact Mass = 2971.5)

*Intermediate **9**:*

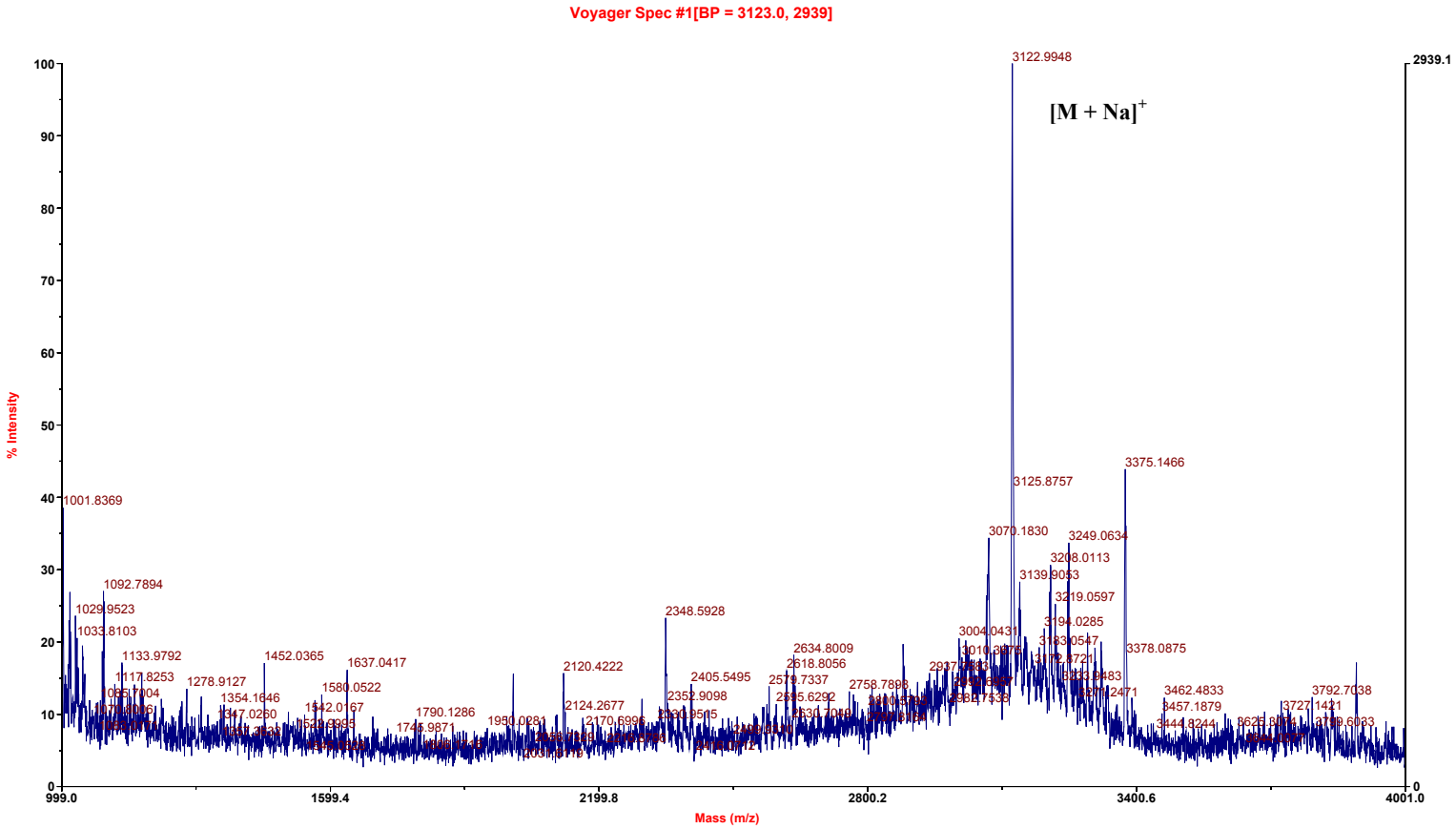


Figure 5 MALDI-TOF spectrum of **9** (crude, Exact Mass = 3096.7)

*Intermediate **10**:*

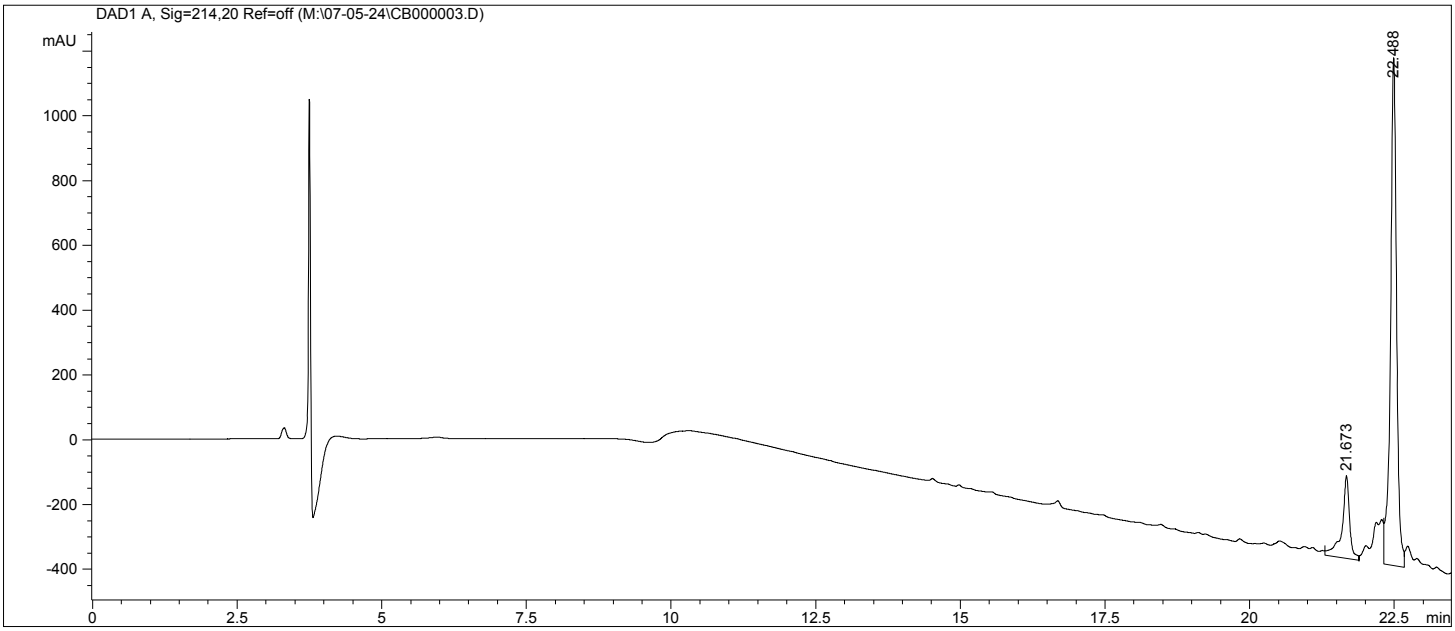


Figure 6a Crude HPLC chromatogram of **10** (Jupiter column) at 214 nm, intermediate **10** is eluting as the major compound at t_r = 22.5 min

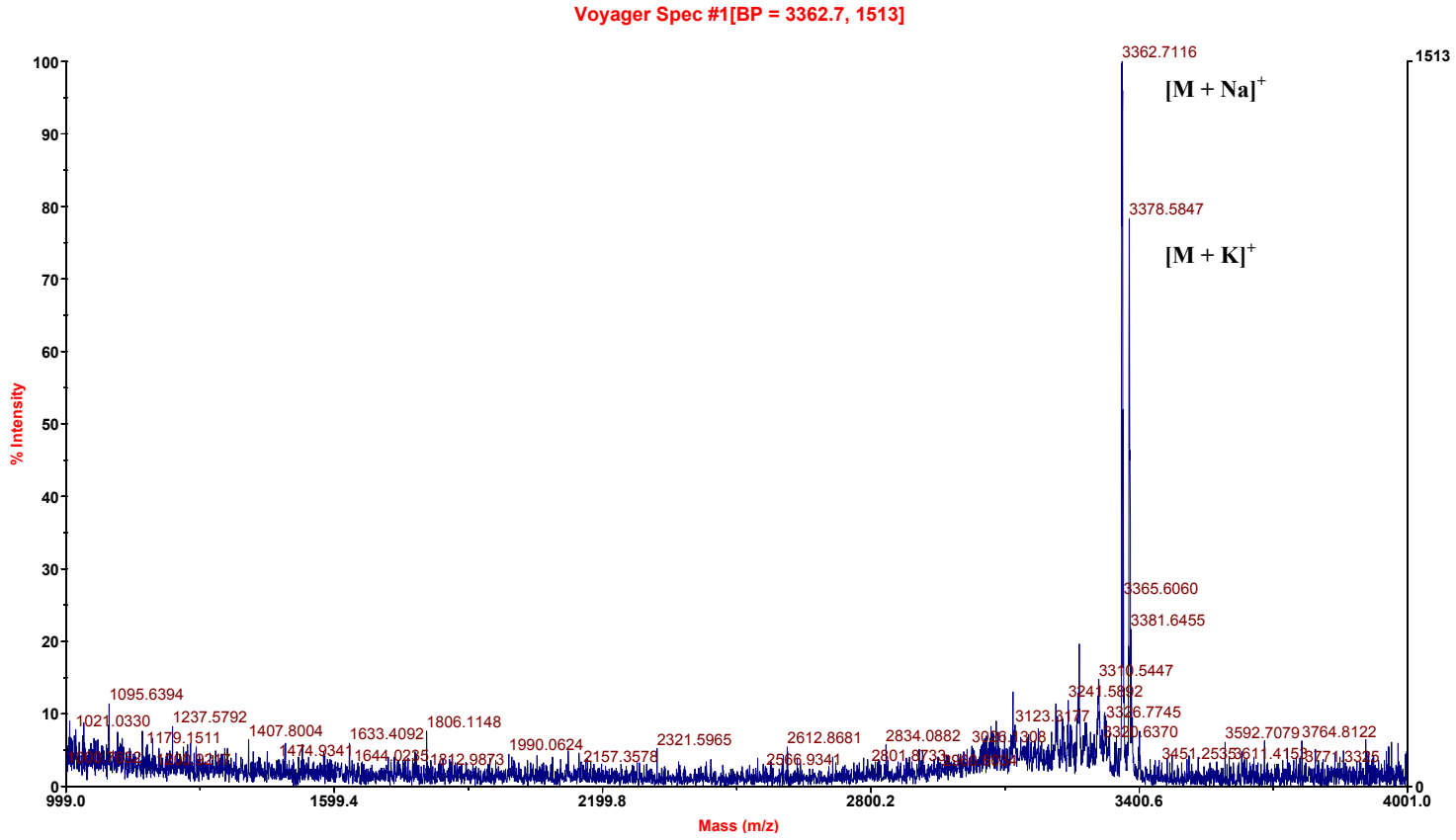


Figure 6b MALDI-TOF spectrum of **10** (crude, Exact Mass = 3337.7)

*Intermediate **11**:*

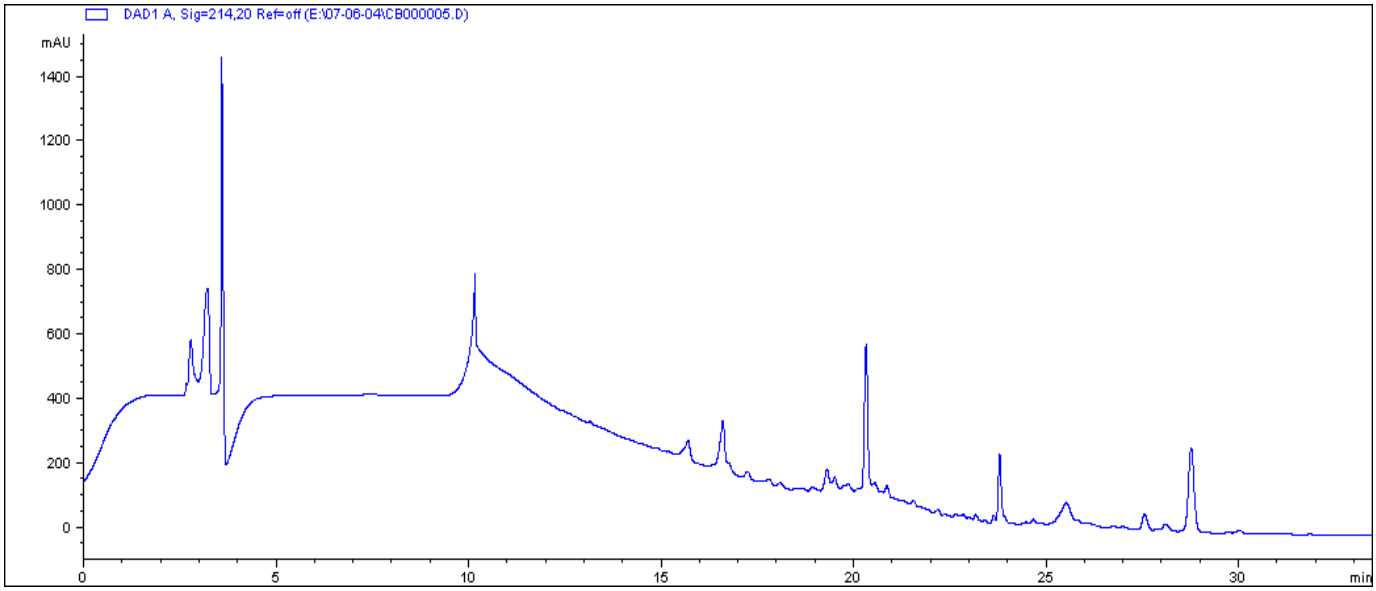


Figure 7a Crude HPLC chromatogram of **11** (Jupiter column) at 214 nm, intermediate **11** is eluting as the major compound at t_r = 20.3 min

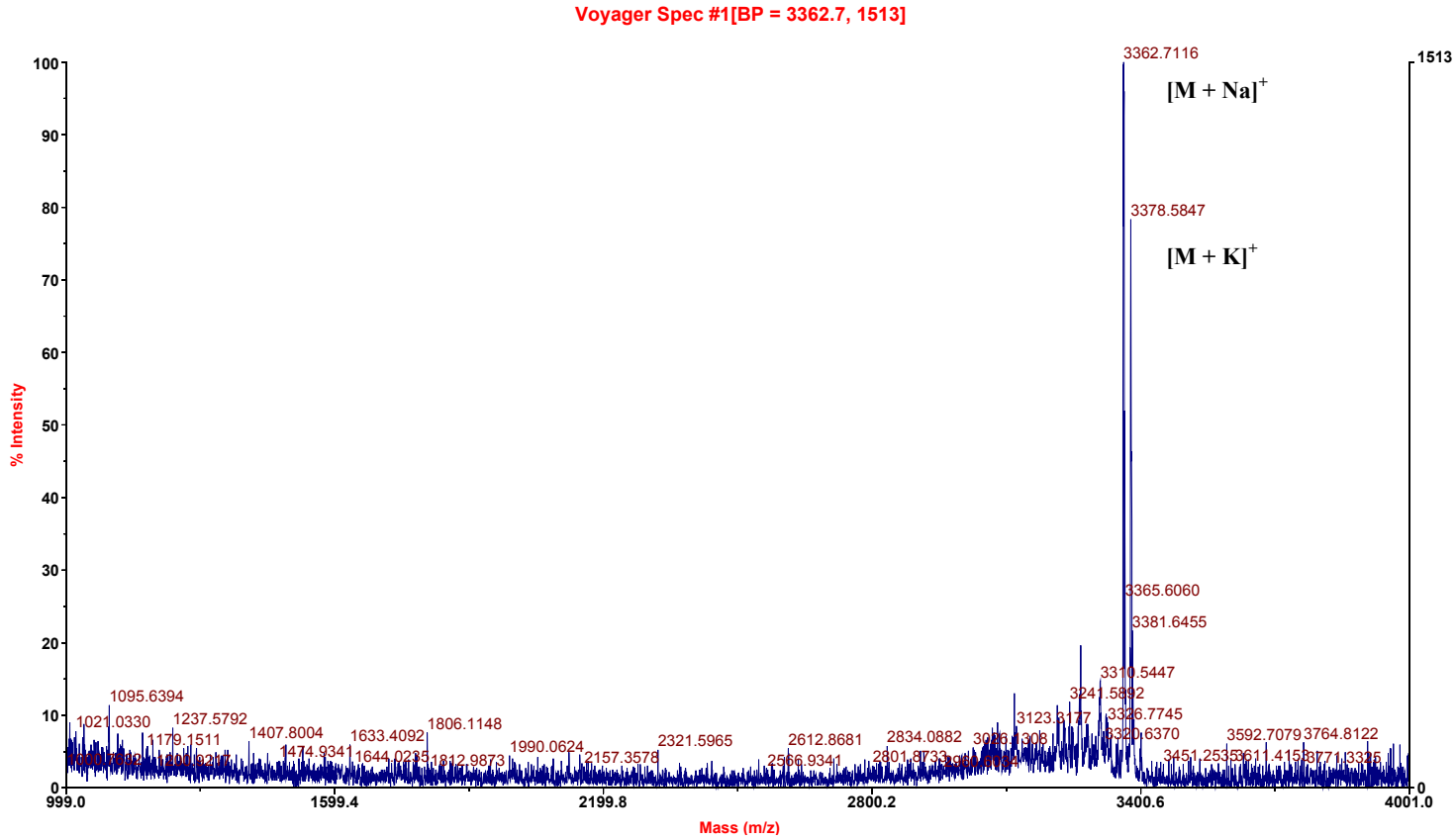


Figure 7b MALDI-TOF spectrum of **11** (crude, Exact Mass = 3337.7)

*Intermediate **12**:*

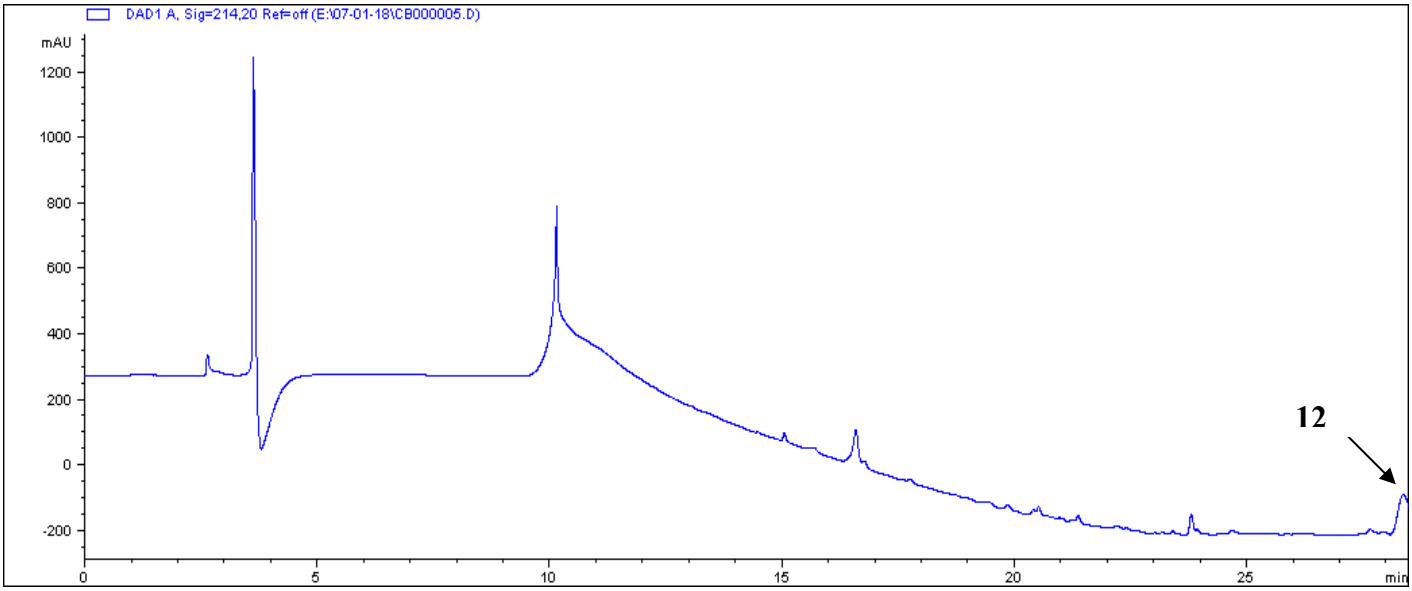


Figure 8a Crude HPLC chromatogram of **12** (Jupiter column) at 214 nm, intermediate **12** can be noticed eluting at t_r = 28.5 min at the end of the HPLC run

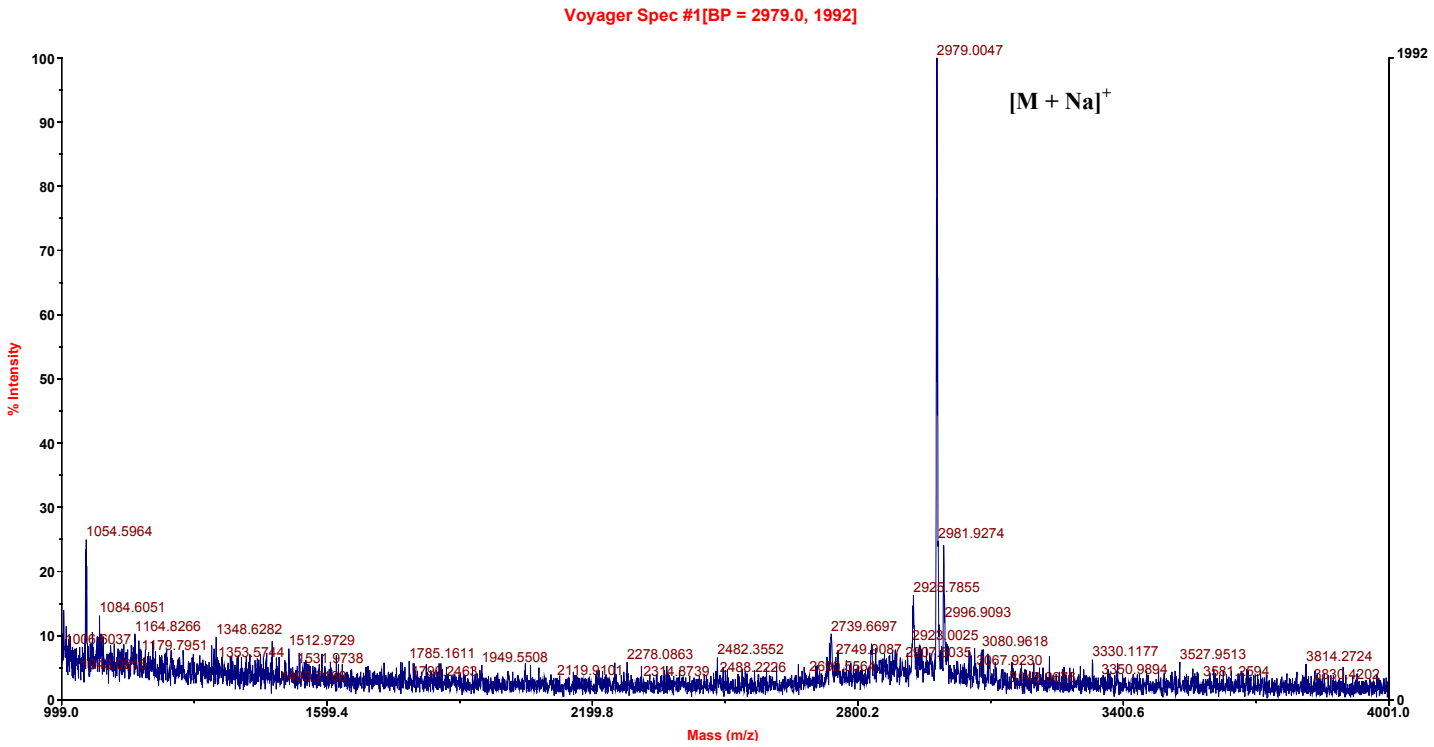


Figure 8b MALDI-TOF spectrum of **12** (crude, Exact Mass = 2953.5)

*Final compound **2**:*

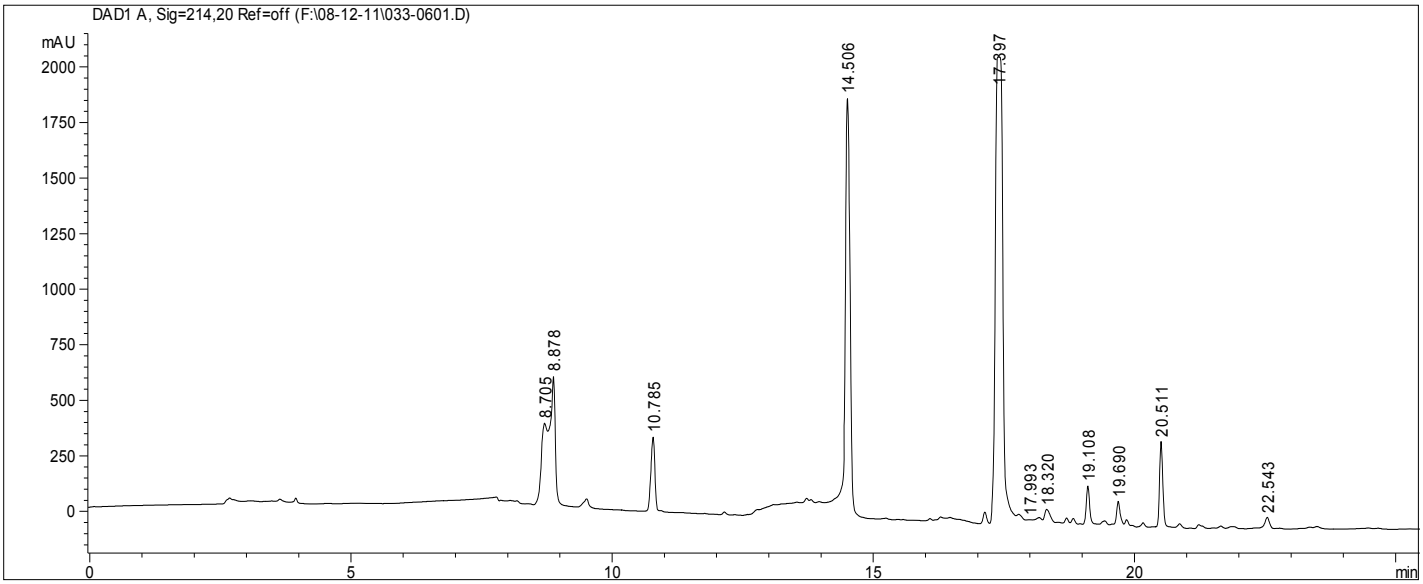


Figure 9a Crude HPLC chromatogram of **2**, compound **2** is eluting at t_r = 14.5 min (protecting group residues give rise to extra peak at 17.4 min as evidenced from their absorbance also between, 254 and 310 nm, these could be removed by purification as described)

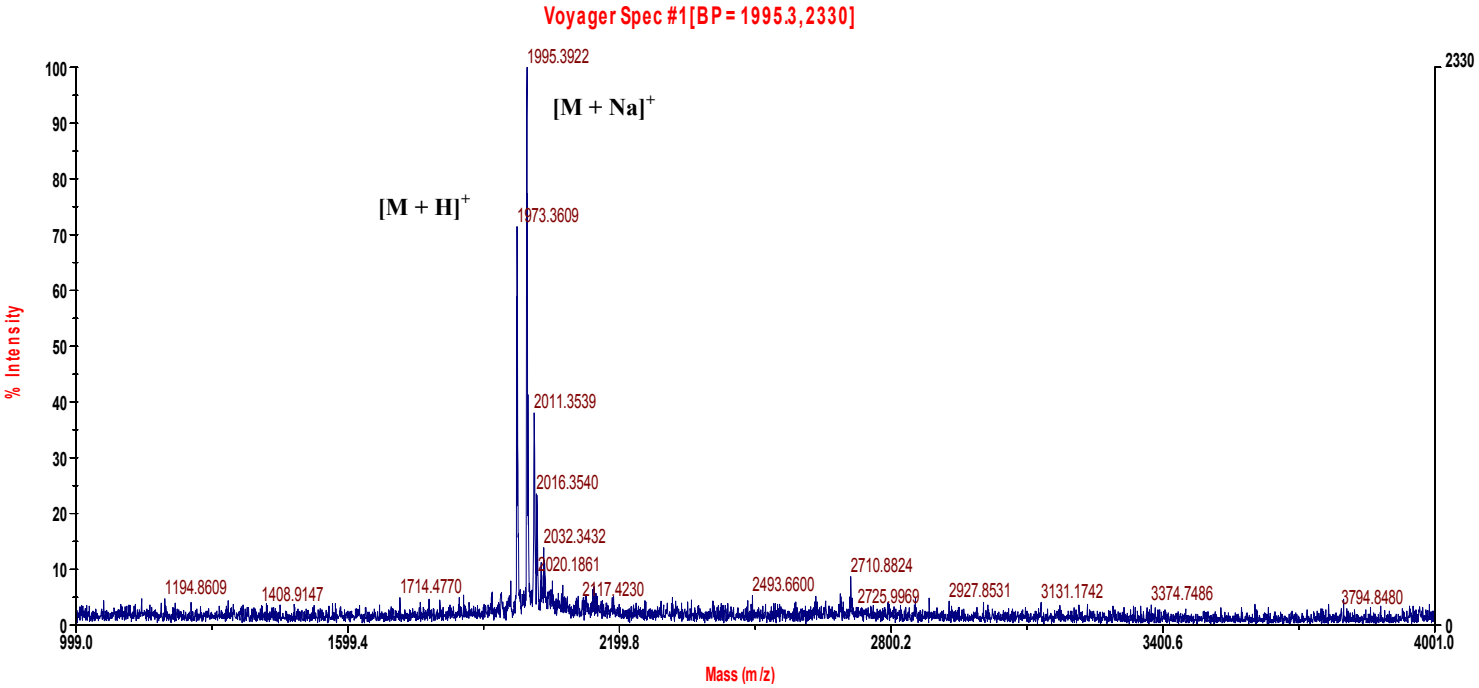
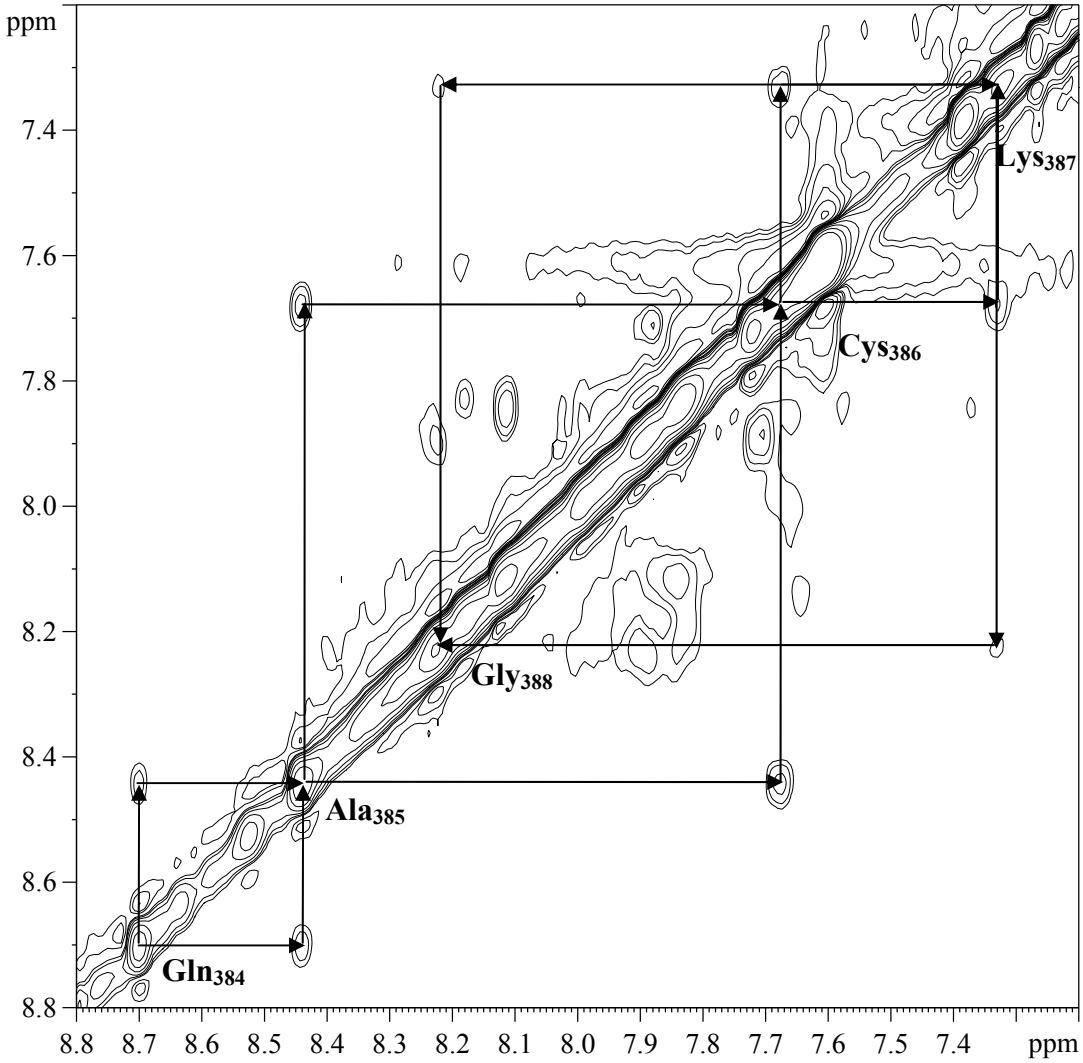


Figure 9b MALDI-TOF spectrum of **2** (crude, Exact Mass = 1971.0)

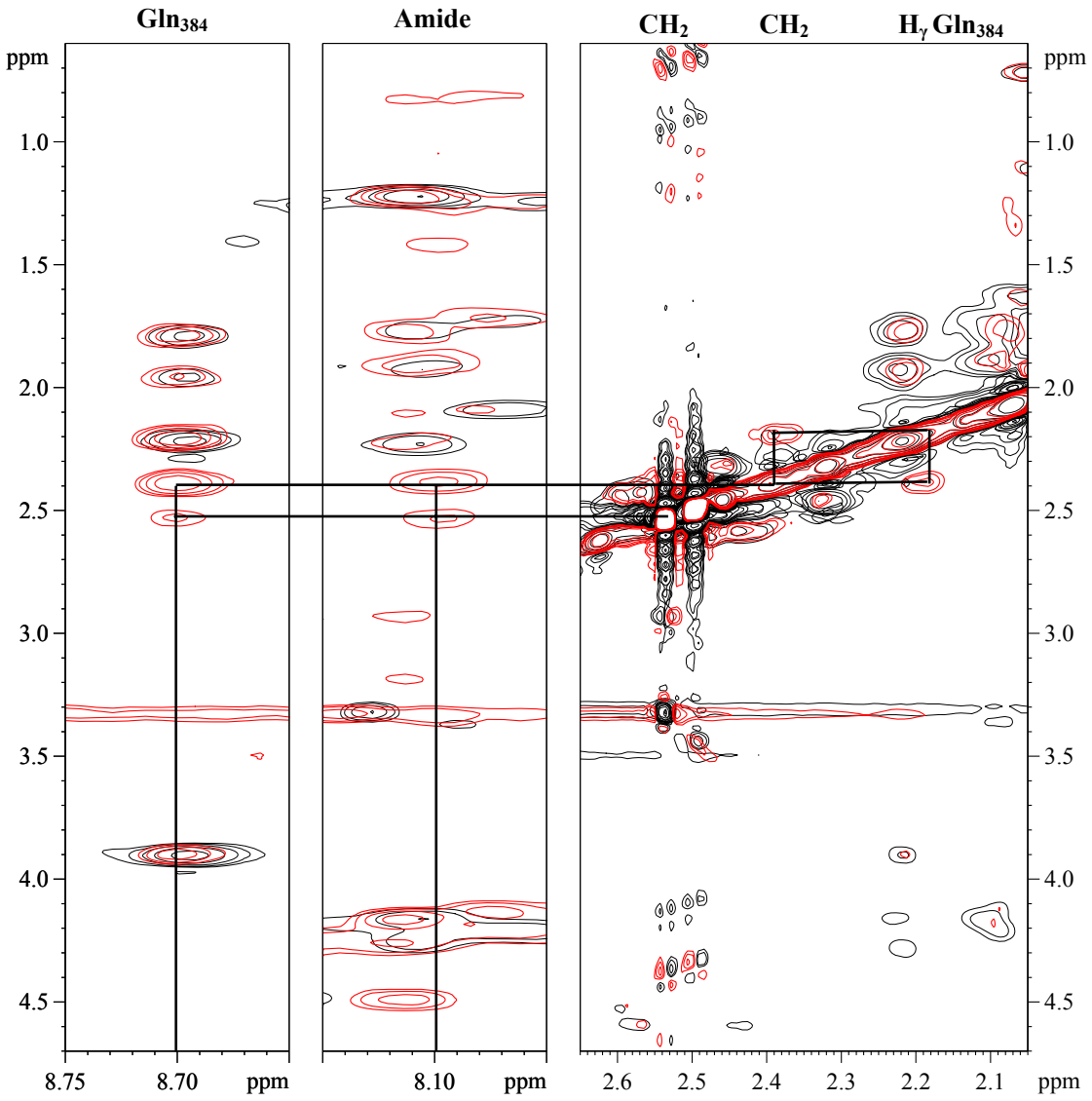
II. 2D NMR study of cyclic peptidosteroid 2

a) *Helical conformation in the Q384-G388 pentapeptide*



Part of the 2D NOESY spectrum with 300 ms mixing time of **2** at 298K and in DMSO-d_6 solution. The sequential assignment of the Q384-G388 is indicated. The presence of moderate to high intensity NH_i-NH_{i+1} nOe's indicates that this part of the peptide chain adopts a conformation in the α-region of the Ramachandran chart.

b) *Correct peptide to scaffold ring closure*



Part of the 2D TOCSY spectrum (mixing time 100ms, black) and the 2D NOESY spectrum (mixing time 300ms, red) of **2** at 298K and in DMSO-d_6 solution. In the NOESY spectrum cross peaks can be observed between the amide proton of Gln384 (8.70 ppm) and two signals (2.53 and 2.38 ppm), coming from the two CH_2 groups that are linking the amino acid chain to the scaffold. Those two signals also show nOe contacts to an amide proton (8.10 ppm) which is not part of an amino acid residue, so can be defined as the amide proton attached to the steroidal scaffold. Together these data provide evidence for the closure from the loop to the steroidal scaffold.

c) Synthesis and analytical data for cyclic control peptide 18

Synthesis of bicyclic peptide 18

Peptide Chain Elongation (14): Resin **13** (100 mg, 0.22 mmol/g) was transferred to an automated peptide synthesiser for chain elongation. For Fmoc deprotection, the reactor was filled with a 0.8-fold coupling volume of a solution of 40% piperidine in DMF and left to react for 3 minutes. The solution was removed by filtration under reduced pressure and deprotection was repeated with a 0.8-fold coupling volume of a solution of 20% piperidine in DMF and left to react for 12 minutes, after which the solution was removed by filtration under reduced pressure and the resin was washed 6 times with DMF (1.1-fold coupling volume) for 30 seconds each. For amino acid coupling, the resin was treated with a solution of Fmoc-amino acid in DMF (0.5M), PyBOP in DMF (0.5M) and DIPEA in NMP (2M). 5 equivalents of amino acid and coupling reagent were used and 10 equivalents of base. The resin was left to react for 40 min. The reaction mixture was removed under reduced pressure and the resin was washed 4 times with DMF (1.1-fold coupling volume) for 30 seconds each. A small amount of resin **14** (2 mg) was cleaved with TFA/H_2O/TIS (95/2.5/2.5) and was used for analytical purposes.

MALDI-TOF: calcd for $C_{68}H_{117}N_{21}O_{22}S_2$: 1643.81, found: 1667.0 $[M+Na]^+$, 1888.2 $[M+Trt+Na]^+$.

Alloc Deprotection (15): Phenylsilane (22 µl, 175 µmol) and $Pd(PPh_3)_4$ (2 mg, 1.75 µmol) were added in this order to a suspension of resin **14** (114 mg, 0.14 mmol/g) in CH_2Cl_2 (0.5 ml) under inert atmosphere. The reactor was immediately flushed with argon and left to shake for 10 min. The reaction was repeated with the same amounts of reagents for 10 min. The resin was then filtered and washed until the resin turned pale yellow. A small amount of resin **15** (2 mg) was cleaved with TFA/H_2O/TIS (95/2.5/2.5) and was used for analytical purposes.

MALDI-TOF: calcd for $C_{65}H_{113}N_{21}O_{22}S_2$: 1603.78, found: 1605.0 $[M+H]^+$, 1848.2 $[M+Trt+H]^+$.

Disulfide Bridge Formation (16): Resin **15** (50 mg, 0.14 mmol/g) was first terminally deprotected. Therefore the resin was treated with a solution of 20% piperidine/DMF (0.5 ml) for 20 minutes. The solution was removed by filtration and the resin was washed once with DMF (1 ml). The resin was treated with a solution of 20% piperidine/DMF (0.5 ml) for 20 minutes. The solution was removed by filtration

under reduced pressure and the resin was washed six times with DMF (1 ml). The resin, previously swollen in DMF, was then treated with a solution of I_2 (18 mg, 70 μmol) in DMF (0.5 ml). The reaction mixture was shaken for 30 min. After reaction, all excess reagents and solvents were removed by filtration under reduced pressure and the resin was washed with DMF (3 x 30 sec, 1 ml), MeOH (3 x 30 sec, 1 ml) and DCM (3 x 30 sec, 1 ml). A small amount of resin **16** (2 mg) was cleaved with TFA/H_2O/TIS (95/2.5/2.5) and was used for analytical purposes.

MALDI-TOF: calcd for $C_{59}H_{101}N_{19}O_{20}S_2$: 1459.69, found: 1482.9 $[M+Na]^+$, 1726.1 $[M+Trt+Na]^+$.

Cyclisation (17): To a suspension of resin **16** (48 mg, 0.14 mmol/g) in DMF (500 μl) were added PyBOP (22 mg, 42 μmol) and DIPEA (15 μl, 84 μmol). The reaction mixture was shaken for 2 h, after which the excess reaction mixture was removed by filtration under reduced pressure and the resin was washed. A small amount of resin **17** (2 mg) was cleaved with TFA/H_2O/TIS (95/2.5/2.5) and was used for analytical purposes.

MALDI-TOF: calcd for $C_{59}H_{99}N_{19}O_{19}S_2$: 1441.68, found: 1442.7 $[M+H]^+$, 1464.8 $[M+Na]^+$, 1480.8 $[M+K]^+$.

Resin Cleavage and Side Chain Deprotection (18): Resin **17** (40 mg, 0.18 mmol/g) was treated with a solution of TFA/H_2O/TIS (95/2.5/2.5) (0.7 ml) for 1.5 h. The solution was removed by filtration, the resin was washed 3 times with TFA (3 x 1 ml) and the combined filtrates were transferred to a centrifuge tube. The TFA was evaporated under nitrogen stream and the product was redissolved in MeOH (0.5 ml) and cooled in an ice bath. To this solution cold ether was added dropwise until the peptide precipitated. The precipitate was centrifuged at 3000 rpm for 5 minutes. The supernatant was decanted carefully and the precipitation and centrifugation steps were repeated twice to give 3 mg of peptide **18** in a crude yield of 30%. Peptide **18** was used without further purification.

t_R: 12.4 min (Jupiter); MALDI-TOF: calcd for $C_{59}H_{99}N_{19}O_{19}S_2$: 1441.68, found: 1464.9 $[M+Na]^+$, 1480.9 $[M+K]^+$, 1486.9 $[M-H+2Na]^+$.

IV. Generation of monoclonal antibodies and ELISA

Monoclonal antibodies BH216, BH21, BH6 and BH195 were harvested from ascites or from hybridoma supernatant produced in a Cell-Line system (Integra, Wallisellen, Switzerland), purified by affinity chromatography using a HiTrap Protein G HP column (Amersham Biosciences, Uppsala, Sweden) and dialysed in a 50 mM borate, 150 mM NaCl buffer (pH 7.5). The concentration was adjusted to 1.55 mg/ml (10 μM).

Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA was performed using a previously described method.[11c] In brief, 96-well plates (Maxisorp, Nalge Nunc, Rochester, NY, USA) were used. Carbonate-bicarbonate buffer (CB) was adjusted to pH 9.6 and washing buffer (WB) contained 154 mM NaCl, 1mM TrisBase (2-amino-2-(hydroxymethyl)propane-1,3-diol), 1.0% Tween-20 (polyoxyethylene(20) sorbitan monolaurate) at pH 8.0. Blocking buffer (BB) was a solution of 136 mM NaCl, 2mM KCl, 15 mM Tris-Acetate (tris(hydroxymethyl)aminomethane acetate), 1.0% BSA, adjusted to pH 7.4. Dilution buffer (DB) was a solution of BB and 0.1% Tween-20. mAb as primary antibody was used in a 1:1000 dilution in DB. As secondary antibody, goat anti-mouse IgG-AP was used (1:750 dilution; Southern Biotechnology, Birmingham, AL, USA) in DB. A 1.35 mM phosphatase substrate (Sigma-Aldrich) in substrate buffer (SB), containing 1 mM AMP (2-amino-2-methyl-1-propanol), 0.1 mM $MgCl_2 \cdot 6H_2O$ adjusted to pH 10.2, was used. Optical density was measured at 405 nm on a SPECTRAmax PLUS^{384} microplate reader system (Molecular Devices, Sunnyvale, CA, USA).

General Washing Step The well plate was submerged in a first batch of WB. The contents was shaken out of the wells and discarded. This washing was repeated twice with the first batch of WB, once with a second batch and once with a third batch. The plate was tapped strongly on paper to discard as much solution as possible.

Coating 50 μl volumes of peptide or protein antigen solutions of varying concentrations (3.2, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, and 0 μM, respectively) in CB were pipetted into separate wells of the well plate. The plate was covered and incubated at 4 °C overnight, followed by a washing step.

Blocking To each well were added 200 μl of BB. The plate was covered and incubated at r.t. for 2 hours, followed by a washing step.

First Antibody Treatment To each well were added 50 μl of a 1:1000 dilution of mAb in DB. For ELISAs with mouse serum, approximately 400 μl of mouse blood were

centrifuged in a 1.5 ml eppendorff tube at 13000 rpm for 20 min at 20 °C. The supernatant serum was decanted and diluted 200 to 145800-fold in DB. 50 µl of serum solution were added to each well. The plate was covered and incubated at r.t. for 90 minutes, followed by a washing step.

Secondary Antibody Treatment To each well were added 50 µl of a 1:750 dilution of goat anti-mouse IgG-AP in DB. The plate was covered and left to incubate at r.t. for 90 minutes, followed by a washing step.

Substrate Development To each well were added 100 µl of 1.35mM phosphatase substrate in SB. The plate was covered and incubated at 37 °C.

Analysis The optical density was measured after 30, 60 and 90 minutes at 405 nm.